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STUDIES IN DOG DISTEMPER.

VI. DOG DISTEMPER ANTISERUM.

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IN 1928 we stated that we had been able to prepare antiserum of considerable potency against the virus of dog distemper, and since that time we have been actively engaged in the preparation of such sera and a study of their properties. In the past numerous sera have been prepared which, it was hoped, would prove of value in stemming the ravages of distemper, but the majority of these have been heterologous sera made by immunising some large animal, such as a horse, with bacteria or bacterial products. We have not performed any experiments with antibacterial sera, as we regarded it as proven that the primary infective agent in dog distemper is the filterable virus of Carré ; it appeared to us to be unreasonable to expect that an antibacterial serum would prove of value in an uncomplicated virus disease, and in the complicated cases it would be largely a matter of chance if the serum we selected contained antibodies appropriate for the secondary bacterial invaders in any given case.

There are, however, three homologous sera on the market at the present time, and they are designed to antagonise the virus infection.

(*a*) The homologous serum of the Cutter Laboratories, U.S.A., is made by hyperimmunising dogs, but there is no published description of the method of its manufacture nor any account of a method

of standardisation.* The manufacturers state that a single dose of from 10 to 20 c.c. of serum will give passive immunity, and that the serum should prove useful in treatment of early cases.

(*b*) Ashe Lockhart, Ray and Barbee (1925) announced the production of a homologous antidistemper preparation (later stated to be a serum), but they gave no information regarding the technique of its manufacture or standardisation. This serum, combined with an antigen (the nature of which was not disclosed), was found by them to give a durable immunity. This double inoculation method of protection was tested later by Skidmore and Buckley (1926), who did not secure such favourable results as the originators of the method.

(*c*) Nusshag and Stecher (1929) published an account of an antidistemper serum prepared in dogs by hyperimmunising with virus and certain unspecified bacterial antigens. No precise record of the hyperimmunising course for the manufacture of the serum is given, and no evidence of standardisation save this : that a dose of 10 c.c. of serum should protect a susceptible dog against distemper infection for a time, and larger doses than this—repeated, if necessary —should prove valuable in the treatment of the disease.

We have not been able to carry out many experiments with these three homologous sera. Dr. Wood, of the Cutter Laboratories, kindly sent us a sample of the Cutter serum, and we have bought further samples of this, and also of the other sera, in the open market. Our experiments, though few in number, have convinced us that none of the samples of serum tested was of high potency, and we judged them to be inferior to the average of our own hyperimmune serum, and much weaker than our best product. We consider it is highly probable that the originators of these three sera encountered the difficulties which we met quite early in our efforts to manufacture a potent serum, and that is the lack of any simple method of testing the serum. Without some reasonably accurate method of assay of the potency of the various sera prepared it is impossible to determine the proper method of manufacture of the serum, and unless some simple and rapid method can be discovered it is impossible to follow the hyperimmunising process intelligently. In agreement with this view is the fact that two sets of workers are satisfied when a dose of 10 c.c. of serum confers passive immunity on a susceptible dog. In our opinion, this is a low standard, for we have records of convalescent sera which reached this level of potency when the serum was harvested at the appropriate time, *i.e.*, about one month after complete recovery from a distemper attack.

It is clear that the ultimate test of a dog distemper antiserum

* Dr. Wood of the Cutter Laboratories informs us in a personal communication that the serum is made by injecting a single massive dose of virus into immune dogs and harvesting the serum " about twelve " days later. Pooled serum from many animals is tested for sterility and from time to time for protective power on susceptible puppies. " Simultaneous injection " of serum (10 and 15 c.c.) and virus is employed. Considerable difficulty is found in securing suitable animals for these tests.

must be carried out on dogs, and, if unlimited susceptible dogs and suitable accommodation for them were available, it would not be difficult, though it would be tedious, to place any number of sera in order of merit. Our own supply of compound-bred dogs has always been strictly limited, and we have been compelled to explore other possibilities of comparison while keeping the dogs as the final court of appeal.

2. *Comparison of the Protective Potency of Immune Sera on Dogs.*

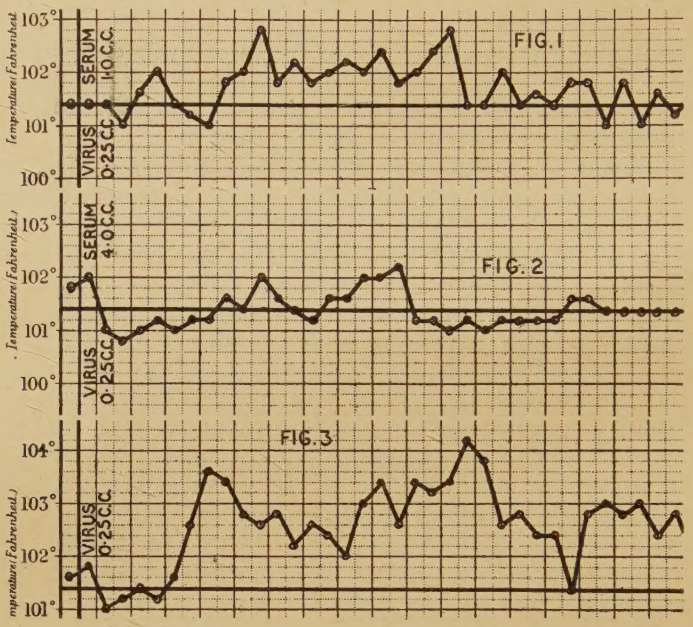
We consider that the protective power of an antidistemper serum can be best estimated in the following manner :—

Dogs of known susceptibility are taken, and a small, but certainly infective, dose of virus is injected into each subcutaneously on one side of the body. At varying time intervals after this assured infection, serum in appropriate dosage is injected subcutaneously on the opposite side of the body. A control dog of the same litter is given virus only. Bi-daily temperature records and clinical notes are kept throughout the whole experiment, and in the course of a week or ten days it is usually clear, by comparing the records of the serum-treated dogs amongst themselves and against that of the control animal, how much effect the serum has produced. When the serum suppresses all signs and symptoms of distemper there can be no question as to the interpretation of the result. When the serum appears to modify the illness there is room for argument as to what conclusion should be drawn. We are, however, strongly of the opinion that a mild distemper attack in a serum-treated dog of our own breeding, with a typical control case, is convincing evidence of an action by the serum and that, other things being equal, the milder the attack the better the serum. We fully realise that this conviction involves the assumption that our puppies, specially bred in strict isolation in the dog compound, if of the same litter, will, in the great majority of cases, give similar and equal responses to equal doses of the same distemper virus. This assumption appears to us to be justified by our general experience with our compound-bred animals, though we are doubtful if the assumption would hold with litters of puppies purchased in the open market. The compound-bred puppy—free from distemper for five or six generations—is invaluable for this class of work.

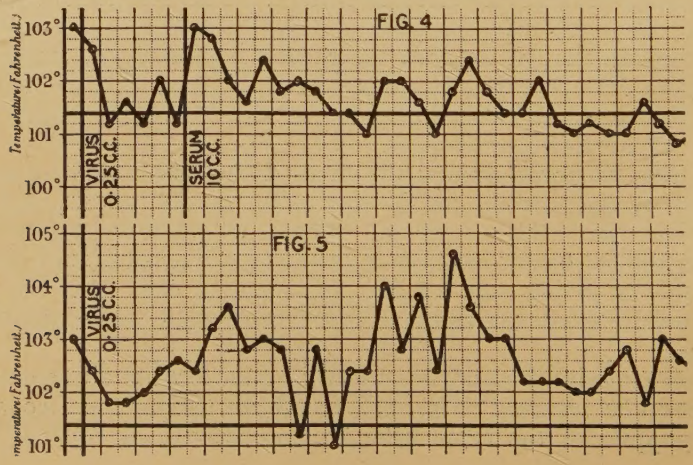
It may be urged against the method that the judgment of the observer is included in the interpretation of the results, and that bias might vitiate the conclusion. That is true, but we endeavour to be just, and are convinced that, in spite of its drawbacks, this method is capable of giving very valuable information, and it has the great merit that it allows an estimate to be made on a small number of animals.

Some experiments may be quoted in brief form to give some idea of the results secured. Figs. 1, 2 and 3 are temperature records of three puppies of the same litter which received an equal dose of

virus. In the case of the puppy of Fig. 1, 1 c.c. of serum was injected on the opposite side of the body a few minutes after the injection of the virus. The puppy of Fig. 2 was treated in the same way, but received 4 c.c. of serum. The puppy of Fig. 3 was the control, and was given virus only.

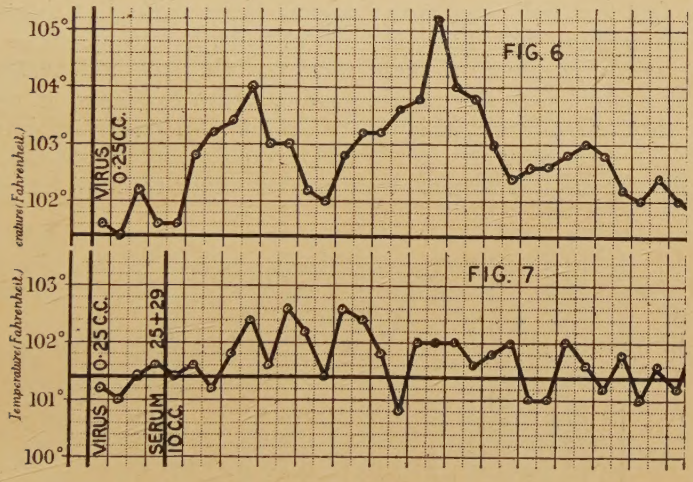


It will be observed that 1 c.c. of serum, administered as described, has almost suppressed the distemper attack, and one dose of 4 c.c. of serum has obliterated every sign of distemper.



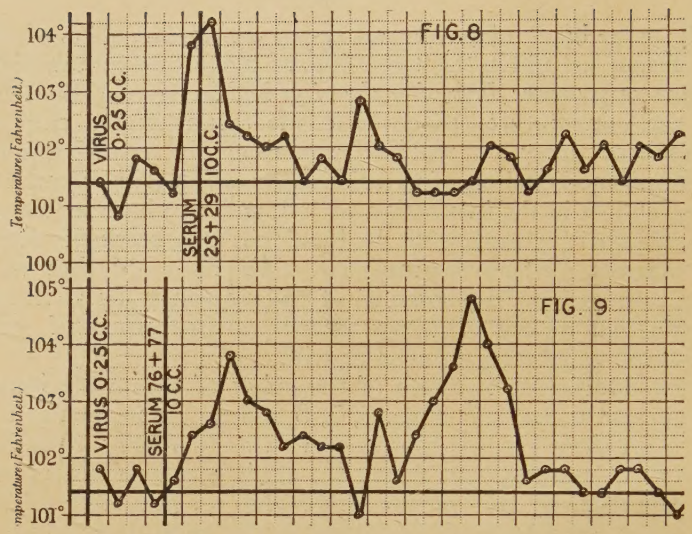
The same batch of serum was tested on two members of another litter of puppies, and in this test a dose of 10 c.c. of serum was injected subcutaneously *three days after the virus had been given.* Figs. 4 and 5 show the temperature responses in the serum-treated and contr animals respectively. It will be observed that the serum has produced a profound modification of the distemper attack, even though it was injected so late in the incubation period, and, putting the five experiments together, it cannot be doubted that this batch of serum has very valuable protective properties. We may add that we have carried out so many experiments of this type with different batches of serum that we are confident that the main features of such test experiments can be reproduced at will.

Some additional examples of the effect of serum are illustrated by temperature charts in Figs. 6, 7, 8 and 9. It seems fair to conclude from these records that serum 76 and 77 (Fig. 9) is inferior to serum 25 and 29 (Figs. 7 and 8). This was amply confirmed by other experiments, and yet serum 76 and 77, in a dose of 10 c.c., will protect completely if it is given at the same time as the virus, but on the opposite side of the body.



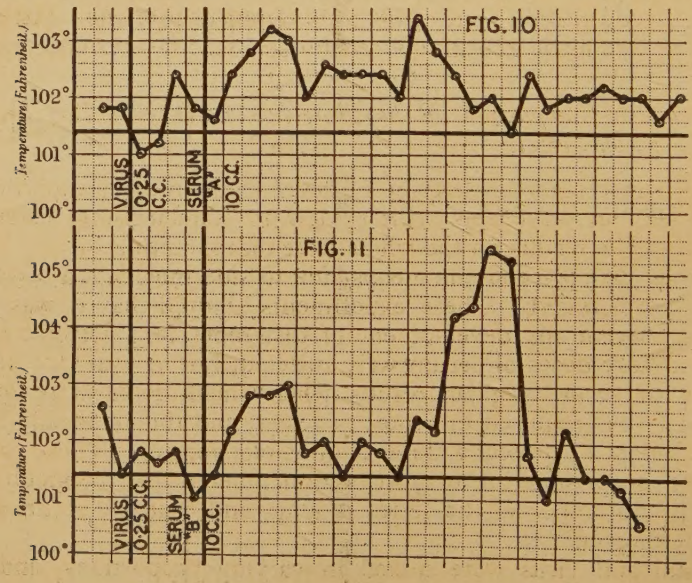
Figs. 10, 11, 12 and 13 show the results with two commercial sera compared with another of our own. Fig. 10, compared with Fig. 13 (the control), shows the modification of the disease which occurred when serum " A " was given in the incubation period. Fig. 11 represents the effect of serum " B " under the same conditions, and Fig. 12 shows that our own serum 26 and 28 has given a much better result than either of the commercial products.

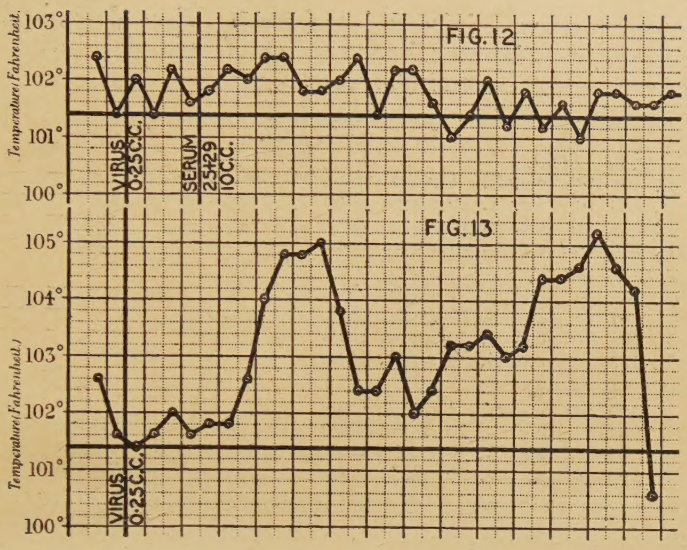
It may be mentioned here that one serum, made from one strain of virus, will protect against viruses from other sources, and we have on occasions shown that one serum will protect against a mixture of virus from three separate sources. We think it unlikely, therefore,



that the comparative failure of the commercial sera can be explained by the fact that they were tested against virus of English origin.

Consideration will show that this method of testing serum would not be perfectly simple if there were no other method available to serve as a guide as to the correct dose of serum to employ in order to secure the desired result, which is the definite and indisputable modification ot a distemper attack. It will be readily understood that in the early days many test animals were wasted either through the dose of





serum being entirely inadequate or else far too large. As the supply of compound-bred dogs is always small—and we insist once more that such animals are essential for this type of work—it became necessary for us to try to secure some alternative method.

Distemper antiserum may also be tested on dogs by mixing falling doses of serum with a constant dose of virus, inoculating the mixtures into susceptible animals, and thus determining how much serum is required to neutralise a given quantity of virus. This method, however, requires too many dogs to be a practicable one. Attempts were therefore made to utilise ferrets for standardisation of dog sera.

3. *Attempted Comparison of the Protective Potency of Dog Immune Sera on Ferrets.*

It is unnecessary to describe in detail the numerous experiments we carried out on ferrets with a view to standardising distemper antisera. The results on the whole were disappointing.

In the first place simultaneous side to side inoculation of serum and virus was tested. These experiments showed that it was possible to prevent the development of distemper in a ferret by this means if large doses of serum were injected, but it was not found possible to secure regular results when small doses were given. About 5 c.c. is required to protect a ferret, and this is usually more than sufficient to protect a three-months old puppy. These results may seem surprising, but it must be remembered that the incubation period of distemper in the ferret is long, and that the serum used was heterologous.

Serum and virus were mixed in varying proportions, and the

resultant mixtures were injected into ferrets. Virus alone, in diminishing dosage, was injected into a series of control animals. The result of such a test is seen in Table I.

TABLE I.
January 5th, 1929.

	Result.
Serum D. 25 and 29.	
F. 192 1·0 c.c. S. 1 mgm. virus	— — —
F. 193 1·0 c.c. S. 10 mgm. virus	— — —
F. 194 0·1 c.c. S. 10 mgm. virus	— — —
F. 195 0·01 c.c. S. 10 mgm. virus	Symptoms 8th day.
Serum D. 31 and 32.	
F. 196 1·0 c.c. S. 1 mgm. virus	— — —
F. 197 1·0 c.c. S. 10 mgm. virus	— — —
F. 198 0·1 c.c. S. 10 mgm. virus	— — —
F. 199 0·01 c.c. S. 10 mgm. virus	Symptoms 7th day.
Normal Dog Serum.	
F. 200 1·0 c.c. S. 1 mgm. virus	Symptoms 7th day.
F. 201 1·0 c.c. S. 10 mgm. virus	Symptoms 7th day.
F. 202 0·1 c.c. S. 10 mgm. virus	Symptoms 7th day.
F. 203 0·01 c.c. S. 10 mgm. virus	Symptoms 7th day.

Nine ferrets were used to titrate the virus alone, and three receiving 0·001 mgm. all sickened and died. Therefore, all ferrets in the above experiment receiving 10 mgm. of virus received 10,000 minimal infective doses, and probably more.

The experiment shows decisively that both immune sera have considerable protective power, and that normal dog serum is worthless. A considerable number of experiments was then put up along similar lines, but it was not found possible to secure constant results, although it was shown again and again that the immune dog serum could neutralise many infective doses of virus. An adequate explanation of the irregularity of the results of these tests has not yet been discovered. Several possibilities have been explored, and we still have hopes that standardisation along these lines may ultimately prove to be feasible. In the meantime we may note that the one significant fact which has emerged from the attempt to standardise dog serum on ferrets is that 0·1 c.c. of a good serum will neutralise 10,000 infective doses of virus.

If the tests of sera on dogs and ferrets had been accurate and easy to perform, they would still not have been exactly the tests we desired. They all occupy too much time. In order to follow properly the production of antibody in a dog during hyperimmunisation, it is necessary to have a test for antibody which gives an answer in a short time, so that the rise or fall of antibody following each injection of antigen can be plotted from day to day.

Efforts were made to devise a rapid method. No skin reaction was available with distemper virus. We could find no evidence of agglutinins or precipitins in hyperimmune serum of excellent quality. Ultimately it was found that a serum of good protective power, as measured on dogs, also possessed the property of fixing complement in the presence of appropriate antigen. This discovery of a test tube reaction allowed almost unlimited tests to be carried out in a comparatively short time, and did not require numbers of susceptible

animals with all the rigid isolation ritual attendant on experiments with such a highly infectious disorder as dog distemper.

It was evident from the outset that this complement fixing property of immune serum was not necessarily associated with protective power, and, even if this could be shown to be the case, it was doubtful if a quantitative relationship could be shown to exist between complement fixing properties and protective power.

However, the possibility that the two properties were related had to be explored, and it may be said at once that we are now satisfied that there is a close connection between the two, though we have been unable to determine that this is a simple quantitative relationship.

4. *Complement Fixation by Immune Serum and Appropriate Antigen.*

Our routine test is carried out by the rapid method as follows :— Serum, antigen, and complement are mixed in small measured doses, in small test-tubes, and the mixtures heated in a water bath at 37° C. for thirty minutes. At the end of this time sensitised red cells are added, and the mixtures incubated for another period of thirty minutes. The answer may be read at once, or the rack of test tubes set aside till the following day. Where complement fixation has occurred there is no lysis of red cells, and where fixation is partial or non-existent there is a varying degree of hæmolysis up to complete lysis.

The Serum.—Dog serum contains complement, and in our routine tests all sera are heated to 56° C. for fifteen minutes in order to destroy the dog complement. Care must be taken not to overheat the serum, as prolonged heating, or a higher temperature than 56° C., is liable to cause loss of the antibody. In our experiments all tests have included a set of tubes with a standard serum, and this is treated in parallel with the unknowns throughout, and unless the standard serum gives an answer of about the expected value the whole test is suspect. The dose of serum employed varies from 0·1 c.c. to 0·01 c.c., according to its potency.

The Antigen.—As antigen we usually employ ferret spleen—fresh, frozen, or dried. In the case of wet specimens, about 2 gm. are weighed out and rubbed up in a mortar with sharp sand. Sufficient saline is added to make a 20 per cent. suspension of the spleen pulp, and the suspension is centrifuged at about 3,000 r.p.m. for ten or fifteen minutes. In the case of dried spleens a 2½ per cent. suspension of the finely ground powder is made in normal saline, and the centrifugalisation is omitted. The usual dose of antigen for each tube is 0·1 c.c.

Complement.—A dilution of guinea-pig serum is employed as complement, and, as a rule, this is freshly prepared for each day's work. In any case the minimum hæmolytic dose is determined, and every mixture of antigen and serum is put up in triplicate with three different doses of complement, either 2, 4 and 6, or 2½, 5 and 10, minimum hæmolytic doses of complement.

As a test object for complement we have always employed 1 c.c. of a 2½ per cent. suspension of sheep red cells, sensitised with hæmolytic amboceptor, prepared in horses, and procured from Messrs. Burroughs Wellcome and Co.

In principle, then, our method is to determine how much complement will be fixed by 0·1 c.c. of whole serum, or a dilution thereof, in the presence of living distemper virus, the whole test being carried out by the rapid method of short incubation at 37° C. It is true that this method does not give the maximum possible fixation, but it avoids certain pitfalls which will be considered later.

Fresh dog serum seems to be devoid of anticomplementary property, and if kept free from bacteria (without antiseptics) in the cold room it remains valid for months.

The antigen is an opalescent or sometimes turbid fluid, but this does not interfere with the reading of lysis if due precautions are taken, and in our experience the fresh antigen is never anticomplementary to any significant degree.

It may be added that stringent precautions are taken to use the cleanest of apparatus and freshly prepared saline, and to control as carefully as possible every reagent used in an experiment.

With the rapid method we have described above we have now performed a large number of tests, and we are convinced that, if the test is kept in its simplest form and broad results only are sought for, it is specific for distemper virus and distemper antibody.

The Effect of Variation of the Antigen.

The following protocol (Table II) is quoted from some experiments which were carried out for the purpose of testing the specificity of the reaction :—

TABLE II.

Serum D. 25 *and* 29. 0·1 c.cm.

	Antigen Rhodes Virus.	*Antigen Higgens Virus.*	*Antigen Normal Ferret Spleen.*	*Antigen Alc. Ext. Distemper Spleen.*
	0·1 c.c.	0·1 c.c.	0·1 c.c.	0·1 c.c.
Complement M.H.D. 2½	++++	++++	0	0
,, 5	++++	++++	0	0
,, 10	++++	++++	0	0
Controls of antigens ,, 2½	0	0	0	0

Control of serum 0·1 c.c. + 2 M.H.D. complement = 0

In this and in all other tables ++++ = complete fixation of complement.
0 = no fixation of complement.
+++, ++, or +, indicates proportional fixation of complement.

It will be observed that two different strains of distemper virus showed striking fixation of complement in the presence of a good hyperimmune serum, and there was no sign of fixation of complement

by normal ferret spleen or with an alcoholic extract of distemper ferret spleen when these were tested against the same serum under the same experimental conditions. It may be added here in amplification of these results that all strains of distemper virus that we have so far tested appear to be the same with the complement fixation test, just as is the case with cross immunisation tests or protective tests with serum. Dog tissue, spleen, lymph gland, brain, or liver which contain distemper virus also form efficient antigens.

Normal ferret liver tissue or lymph gland appeared to have no antigenic property under these conditions of experiment. Some dog tissues may show traces of non-specific complement fixation, but the degree of fixation is so small that it does not give rise to any serious error by our standard method of test.

The importance of a high virus content in the antigen is shown by dilution of an efficient antigen. In the protocol in Table III it will be observed that the fixation results soon diminish with dilution of the antigen, and by the time a 1 per cent. suspension of spleen is reached the fixation is becoming poor. It is noteworthy that this percentage of spleen contains many infective doses of virus per cubic centimetre, and therefore it is clear that this rapid method of complement fixation will only demonstrate large amounts of virus.

TABLE III.

Serum D. 25 *and* 29. 0·1 *c.c.*

	Gland Antigen 4%	*Gland Antigen* 2%	*Gland Antigen* 1%
	0·1 c.c.	0·1 c.c.	0·1 c.c.
Complement M.H.D. 2½	++++	++++	++++
,, 5	++++	++++	++
,, 7½	++++	++++	0

Antigen control 0·1 c.c. 4% A. with 2½ M.H.D. complement = 0
Serum control 0·1 c.c. S. with 2½ M.H.D. complement = 0.

We are convinced from these and other closely related experiments that with a good serum it is possible to estimate roughly the virus content of a tissue (spleen, lymph glands, liver, or brain), provided that only large amounts of virus are taken into consideration. We now rely on this test, and use it when we wish a rough and rapid assessment of the virus content of a tissue which we propose to use for an experiment, such as, for example, hyperimmunisation of dogs. In Table IV it will be seen that a 5 per cent. and 1 per cent. suspension of certain dog tissues (spleen and lymph gland mixed) gave quite good fixation of complement with our standard serum. This tissue was therefore used to hyperimmunise four dogs with an excellent result.

When we were considering the manufacture of vaccine (1928) we stated that it was our custom to prove the virus content of all tissue included in a vaccine by demonstrating that small doses of the

TABLE IV.
Serum D. 17. (*Standard Serum*) 0·1 *c.c.*

	Antigen 20%.	*Antigen* 5%.	*Antigen* 1%.
	0·1 c.c.	0·1 c.c.	0·1 c.c.
Complement M.H.D. 2	++++	++++	++++
,, 4	++++	++++	++++
,, 6	++++	++++	+++

Antigen control 0·1 c.c. 20% Antigen + 2 M.H.D. complement = 0
Serum control 0·1 c.c. + 2 M.H.D. complement = 0.

tissue were infective for ferrets. This occupied much time, and we believe that this newer serological method should prove of great assistance in vaccine production, for the desired test can be made and the answer secured rapidly, without animal experiment.

Again, we have found this test useful in post-mortem examinations of dogs which are reputed to have died from distemper.

So far as our experience goes, it may be said that any tissue from dog or ferret which shows good complement fixation with our standard serum (by this technique) contains plenty of distemper virus. Such material has been shown on occasions to be infective for ferrets and on others to be an adequate stimulus in the hyperimmunisation of dogs.

We have found that heating the spleen emulsions to 50° C. for an hour seriously impairs, though it does not destroy completely, the antigenic property of the emulsion, and heating to 60° C. for thirty minutes caused total destruction of the antigenic property. The loss on even gentle heating is so serious that we find it difficult to reconcile our results with those of Bullier (1929), who found that fixation of complement might occur when serum from dogs attacked by distemper was allowed to react with a distemper brain antigen which had been heated " to 100° for half an hour."

Formaldehyde (up to 1 part in 1,000) was added to some of our antigens in the hope of securing a stable product which could be kept as a standard, but it was found that, although the formalin had no immediate deleterious action on the antigen, with the lapse of a few days deterioration might set in, and frequently the formalised antigen was of quite poor quality after one week. In other specimens the value of the antigen persisted for longer than this but ultimately disappeared. This was a disappointment to us, as, in addition to the hopes of a stable antigen, we had visions of a serological test which could be used to control the value of formalised vaccines. It is now clear that the antigenic property required for the complement fixation test disappears long before the vaccinating potency has seriously diminished.

The addition of cholesterol to the antigen was tried in the hope that this might increase the sensitivity of the reaction, but this hope proved vain.

Summarising our experience, we may say that one essential reacting constituent in the complement fixation test appears to be distemper virus.

The Effect of Variations in the Serum.

In exploring the effect of alterations in the antigen in the results with complement fixation we always used an excess of the best quality serum which was available, and, conversely, when studying the relative potency of different sera we have employed a full dose of the most potent antigen which we could secure at the time. We were very fortunate in securing, quite early in the work, one specimen of serum (D. 25 and D. 29) which gave excellent fixation results when used with appropriate antigen. As a preliminary, therefore, all the available sera were compared against this specimen. Later on, we chose as a standard one serum (D. 17), which proved to be potent in protecting dogs, and have compared all sera with this one in the numerous tests we have carried out during the last twelve months.

In the first place, normal dog serum and normal ferret serum show no fixation of complement by this technique. Convalescent serum shows only traces of complement fixation under conditions in which immune serum gives strong positive reactions. The various sera prepared by hyperimmunising dogs gave a certain amount of fixation in every instance, and the degree of fixation given by any one serum appears to be regular and constant in relation to the standard serum D. 17.

Table V shows the answer usually obtained with standard serum (D. 17) and that secured with one of our more potent immune sera (D. 33). Convalescent serum at five times the concentration of the standard serum will not show the fixation of two minimal hæmolytic doses of complement by the same technique.

TABLE V.

Ferret Spleen Antigen 20% 0·1 *c.c.*

	D. 17			*D.* 33.		
	0·02 c.c.	0·04 c.c.	0	·005 c.c.	0·01 c.c.	0·02 c.c.
Complement M.H.D. 2	++++	++++		++	+++	++++
„ 4	+++	++++		0	+	++++
„ 6	0	++++		0	0	++++

Serum controls D. 17 0·04 c.c. + 2 M.H.D. complement = 0
D. 33 0·02 c.c. + 2 M.H.D. complement = 0
Antigen 0·1 c.c. + 2 M.H.D. complement = 0

The second reacting constituent in our test appears to be distemper antibody.

5. *Some Limitations of the Complement Fixation Test.*

From time to time we have endeavoured to make the complement fixation test more sensitive. We were disappointed that the convalescent sera gave such poor results, for we knew, from tests on dogs, that such sera had definite protective powers. Moreover, we considered that if the sensitivity could be greatly increased without loss of specificity it might be possible to elaborate a test which could be relied on to pick out a dog that was " over distemper " from amongst a group of susceptible animals. The most obvious step to take was to increase the time which was allowed for the reaction between the

antigen and the antibody in the presence of the complement. We have tested incubation of the mixtures for one hour instead of the standard thirty minutes, holding the mixtures at room temperature or in the ice box for twenty-four hours ; and incubation for half an hour followed by maintenance at room temperature overnight. All these procedures greatly increase the number of units of complement which are bound by immune serum, and will also demonstrate fixation of complement by convalescent serum. The drawback to such procedures is that the controls with normal serum do not remain clean. Normal dog serum in the presence of distemper virus obtained either from ferret or dog spleen will fix an appreciable amount of complement and, on occasions, as much as is bound by the serum of a recovered dog. In Table VI are shown the results of one experiment where the mixtures were incubated for half an hour, and then kept at room temperature overnight. The normal dog sera (N.D.1, 2, 3, 4) were obtained from " compound " dogs, and there can be no question as to the distemper susceptibility of these donors. Tests were also put up with horse, sheep, hog, goat, and ox serum. Only the results of the horse and sheep are shown, as goat, hog, and ox serum gave essentially the same result as the sheep serum.

TABLE VI.

Antigen Ferret Spleen 20% dose 0·1 c.c.

	D. 17	*D.* 17	*N.D*$_1$	*N.D*$_2$
	0·01 c.c.	0·02 c.c.	0·1 c.c.	0·1 c.c.
Complement M.H.D. 2	++++	++++	++++	++++
,, 4	++++	++++	++++	++++
,, 6	+++	++++	+++	++++
,, 8	0	++++	+	+++
,, 10	0	++++	0	+
Serum control M.H.D. 2	0	0	0	0

	N.D$_3$	*N.D*$_4$	*Horse.*	*Sheep.*
	0·1 c.c.	0·1 c.c.	0·1 c.c.	0·1 c.c.
Complement M.H.D. 2	++++	++++	++++	++++
,, 4	++++	++++	++++	++++
,, 6	++++	++++	++	++++
,, 8	++++	+++	0	++++
,, 10	++	++	0	++++
Serum control M.H,D. 2	0	0	0	0

Control of Antigen 0·1 c.c. + 2 M.H.D. = 0.

It may be mentioned that two of the above normal dog sera were re-tested by the rapid method, using dog antigen, and no appreciable fixation of complement occurred. On this occasion horse serum also gave a negative result, but sheep and goat serum fixed two minimum hæmolytic doses of complement.

We believe, from the above and other experiments, that non-specific fixation of complement, though it is an interesting pheno-

menon, will not cause error if our standard method of performing the test be adopted.

6. *The Manufacture of Potent Dog Distemper Immune Serum.*

Having determined the essential facts regarding the fixation of complement by distemper virus and immune serum of good quality, we felt in the position to study the development of antibody during the course of immunisation. For this purpose dogs that had recovered from a distemper attack or been immunised were injected with massive doses of distemper virus in a variety of ways. The number of the injections was varied, and the times between the injections were made long or short. At intervals small samples of blood (1 or 2 c.c.) were taken from the external saphena vein by means of a syringe and sharp hypodermic needle under full aseptic precautions. In our earlier experiments whole serum was used for the tests, but recently the measured volume of blood was ejected into four volumes of saline and tests were carried out on the dilute serum which was separated from the clot by centrifugalisation.*

It was early evident that the injections of virus into a dog in a haphazard manner did not give good results, and that the number of hyperimmunising doses was no guide to the value of the final product. We found that some dogs which had given a good response to the first hyperimmunising dose of virus deteriorated after further injections. This fact is, we believe, the principle reason why we failed to secure an antiserum when hyperimmunising dogs in 1925. At that time also we knew very little about the technique required for demonstrating and estimating the amount of protective property in a serum.

It was clear, also, after a time, that the rise of antibody following a massive injection of virus might be very rapid, attain a maximum quickly, and then subside again as rapidly as it rose. We, therefore, found it necessary to follow the antibody in the dog very closely, and when we believed it to be maximal we considered it advisable to exsanguinate the animal under full anæsthesia.†

We have found the following procedure gives, on the whole, the best value antiserum :—

Dogs which have recovered from distemper attacks, or, what we consider preferable, dogs that have been artificially immunised, are kept for at least a month after all symptoms have subsided. A supply of virus is secured (and this should be as potent as possible) from an acute, uncomplicated, case of distemper in a dog. Twenty c.c. of a 20 per cent. suspension of this virus (spleen and lymph glands) are

* The blood is thus diluted 1 in 5 and the serum is calculated to be 1 in 10, for the red cells account for approximately half the volume of the whole blood. The calculated dilution is not accurate but we believe it to be near enough for our purpose.

† The dogs are anæsthetised with chloroform followed by C.E. Mixture. The blood is withdrawn through a glass cannula, inserted into the carotid artery, and when the flow has almost ceased a litre of normal saline is put back into the circulation *via* the jugular vein. After two or three minutes a second and final bleeding is made from the carotid artery. The dilute blood is kept separate from the first, and the dilute serum which separates from the clot is subsequently concentrated.

then injected subcutaneously or intramuscularly, using the strictest aseptic precautions, into the dog it is desired to hyperimmunise. On the following day another dose of virus of the same size is injected on the side of the body opposite to the first injection. There may be some slight local reaction to these injections and a small rise of body temperature. These, when they occur, augur well for the response in the recipient. Abscesses should not develop. On the third, fifth, and seventh days following the second injection, samples of blood are taken and tested for complement fixation. Sometimes on the third, and nearly always on the fifth day, an abrupt rise in antibody will be detected, and this may be maximal on or about the seventh day.

It is impossible to lay down definite rules, as the response of different dogs is variable, but with this method of immunisation the maximum is usually about the seventh day, though it may occur earlier or later than this. We are satisfied if the test sample appears to be distinctly better than our standard, and pleased if it is twice this value.

We have obtained good serum in response to a single hyperimmunising dose of virus, and also to two doses of virus at intervals of a week. In these cases the rise of antibody was slower, and took about ten or fourteen days to reach a maximum. We prefer the method first described.

We are convinced that a long course of virus injections is inadvisable. The total amount of virus that can be injected into a dog is very small compared with the amount which may exist in a dog at the height of a distemper attack, and we feel it is impossible in the present state of our knowledge to thrust upon a dog a really large stimulus to antibody formation. It is better to keep the antibody factory at rest for a time, and then stimulate as suddenly as possible with as powerful a stimulus as can be managed. The suddenness of the stimulus on a resting mechanism compensates to some extent for its small absolute size.

7. *The Fractionation and Purification of Distemper Antiserum.*

The fractionation of distemper antiserum was undertaken with the complement fixation test as a guide to the activity of the fractions secured. It was soon evident that the whole of the activity was precipitated along with the serum globulins. The addition of an equal volume of saturated ammonium sulphate or anhydrous sodium sulphate to the extent of 20 per cent. at 30° C., to immune serum, precipitated all the activity. The further segregation of the most potent fraction of the globulin precipitate did not prove to be a simple matter. In some experiments the activity appeared in the euglobulin and in others in the pseudoglobulin fractions. For example, when the globulins precipitated by sulphates were dissolved, and the resultant solution was saturated with sodium chloride, the activity was precipitated along with the euglobulin. When immune serum

was dialysed free from salts and carbon dioxide passed through the serum, to aggregate the euglobulin precipitate, the activity was found to be in the filtrate along with the pseudoglobulin. After considerable study it became clear that the distemper antibody was associated with a protein which is insoluble in water, and has an isoelectric point about *p*H 5·8. The protein is soluble in weak acids or alkalies, and will dissolve in water saturated with carbon dioxide. From the acid solution it is precipitated by the cautious addition of weak alkali or the removal of the acid. It is soluble in 0·9 per cent. sodium chloride, but not with great freedom, and shows signs of precipitation when such a solution is cooled to 0° C. Free solution with greater stability is secured by means of 3 per cent. sodium chloride. It will be observed that the distemper antibody behaves like the pneumococcus antibody described by Felton (1925, 1926, 1928), and the meningococcus antibody described by Goodner (1930).

The method we have adopted for the preparation of a purified and concentrated antibody is similar to that of Felton (1928), though modifications are required to suit the differences which dog serum presents as compared with horse serum. It is not yet certain that this method is the best possible, and the yield is not very good, but the final product is certainly an elegant preparation.

Method.—Dilute serum is taken and warmed to 40° C. Anhydrous sodium sulphate is added to the extent of 20 grm. for every 100 c.c. of fluid. A precipitate forms at once. The temperature is maintained at or about 40° C. for one or two hours, during which time the fine protein precipitate of the mixed globulins aggregates into visible particles. The precipitate is filtered off, in a hot room, at 37° C., on large Buchner funnels, with the aid of a water pump. It is important to use very low pressures in the filtration, as the precipitate jams if high pressures are employed. When filtration is nearly complete the maximum pressure that can be obtained by the pump is used, and the precipitate is sucked as dry as possible. The cheesy precipitate is removed from the filter papers, rubbed up in a mortar with a little water, and the contents of the mortar transferred to collodion sacs which have been equilibrated against 75 per cent. to 80 per cent. alcohol. The sacs are mounted on rubber corks armed with U-tubes containing mercury.* The sacs are plunged in warm distilled water, which is changed frequently for three days.

Dialysis is continued until there is no more than a trace of sulphate left, and at this time there should be a copious precipitate in the sacs. The contents of the sacs are then collected, and the reaction adjusted to about *p*H 4·8 by the cautious addition of acetic acid. As indicator of reaction we use methyl red and employ the drop method of Felton (1921). A fine and sticky precipitate is present at this stage, and

* This is the well recognised procedure to prevent over dilution of the fluid in the sacs. As water is sucked into the sac by osmosis the pressure inside the bag rises and the imbibition of water is correspondingly checked. Provided the collodion used for the sacs is of good quality and the bags are thick, there is little chance of rupture occurring.

we have usually added 1/40 molecular sodium nitrate in order to assist the aggregation of particles, as was recommended by Felton. This precipitate is filtered off, again avoiding anything more than minimal pressures. The precipitate is dissolved in saline, and forms fraction 1 (Euglobulin). The filtrate is collected, and the reaction adjusted by the cautious addition of dilute caustic soda, to *p*H 5·8 (almost a clean yellow colour with methyl red and a pale dirty green with brom cresol purple). An opalescence forms. Four volumes of distilled water are added, and a precipitate forms at once. It is inadvisable to attempt filtration immediately, as this precipitate is exceptionally sticky, and the material should be held at 0° C. overnight. On the following day the precipitate will be found to have settled completely on to the bottom of the container, and stuck there. A clear supernatant fluid can usually be decanted without filtration. The precipitate on the walls of the container is washed once with distilled water, and then dissolved in 3 per cent. sodium chloride. This represents fraction 2, and should contain the bulk of the original activity. The final filtrate, which is now of considerable bulk, contains the pseudoglobulin, and possesses some activity, but the potency of fraction 2 compensates for considerable loss.

In two successive experiments we secured 50 per cent. and 60 per cent. of the activity in Fraction 2, and the resultant solution was a limpid, faintly opalescent, almost water-clear fluid showing five times the complement fixing power of our standard serum, and we have no doubt that this potency could have been increased five-fold again, if we had so desired, by dissolving the precipitate in a smaller volume of saline.

Hartley (1914) found that the antibody for Rinderpest was precipitated by dialysis or by saturation with sodium chloride, and concluded that the antibody was associated with the euglobulin fraction of ox serum. The effect of small variations in hydrogen ion concentration on the solubility of the euglobulin secured was not investigated. Henseval (1919) found the neutralising property of antivaccinia serum distributed amongst all the proteins of the serum, but secured a potent euglobulin fraction. Weyer, Park and Banzhaf (1929) localise the antibody of poliomyelitis antiserum in the pseudoglobulin fraction. Morgan and Fairbrother (1930) found that the euglobulin faction of poliomyelitis antiserum was the most potent, but describe " general distribution of antibody throughout the serum proteins." It must always be remembered that it is very difficult to separate serum proteins in pure form, and our tests for antibody have been designed to exclude small degrees of activity ; we are satisfied that the bulk of distemper antibody is accounted for by the special globulin fraction we have described.

8. *Protective Potency Compared with Complement Fixing Power.*

We are unable to state categorically that the complement fixing power of a serum and its protective potency are directly associated, but we have the very definite impression that the two properties are

intimately connected. Comparisons of the various sera and their separated constituents have been made on dogs by the method outlined in Section 1. This has enabled us to determine broad differences but has not allowed us to allot precise figures for protective power, and consequently just comparison has been difficult.

From the first, serum D. 25 and D. 29, which gave excellent results in the test-tube, also gave very good results in protecting dogs, and the sera which gave poor fixation results appeared to be inferior in protective powers (D. 76 and D. 77). We may say that we have never yet found a serum which was rich in complement fixing properties which was not also highly potent in protecting dogs, and our impression is that the higher the complement fixing power the more protective the serum will prove to be. On the other hand, it must be borne in mind that there are samples of convalescent sera which give trivial results with the complement fixation test—as carried out by the rapid method—and yet these same sera have very definite value as protective agents.

In the absence of exact figures for protective potency and maximum values for complement fixation tests, accurate comparison is almost impossible, and yet after reviewing our experience an impression remains that the two properties are connected together.

When attention is turned to purified and concentrated antibody, we again find broad agreement between fixation and protection. For example, in one experiment all the complement fixing antibody was accounted for by the mixed globulins, and the greater part of this was found to be associated with the pseudoglobulin fraction, and only a little with the euglobulin fraction of the serum proteins. The protective property was distributed in a precisely similar manner. The mixed globulins proved as good as the whole serum in corresponding dosage, and the pseudoglobulin accounted for the greater part of the activity of the mixed globulins.

More recently, by employing Felton's technique, we have divided the globulins differently, and we have secured a small fraction with strong complement fixing properties. In the single experiment performed this purified antibody appeared to have approximately the protective powers expected, and, as will be seen later (Section 9), it is shown to possess curative properties.

It may be said, therefore, that tests on dogs have not yet proved that there is a direct relationship between the protective power and the complement fixing power of purified and concentrated antibody, but, on the other hand, there is nothing really inconsistent with this view. We feel that it is very significant that the separation of one small fraction of a serum so as to secure richness in one property has not led to serious diminution in another property, and we are of the opinion that the two properties may some day be shown to be associated with a single constituent of the whole serum.

9. *Some Experiments on the Utility of Potent Hyperimmune Serum and the Purified Antibody.*

Our experiments have been directed along three main lines :—

(1) Temporary protection (passive immunity) ;
(2) Durable protection by simultaneous, side to side, inoculation of serum and virus ; and
(3) Treatment.

Passive Immunity.—From the experiments which have been quoted in Section 1, it is obvious that a full dose of good hyperimmune serum will confer immediate passive immunity, and it remained for us to determine how long such an immunity might be expected to last. We did not anticipate that the passive immunity would last for long but we were a little surprised at the short duration of the protection. We found, for example, that a dog which had received twice the dose of serum which conferred immediate and complete immunity on his litter brother became sensitive to a test dose of virus after nine days. The response to virus was a modified one, but quite definite. Two other members of the litter which had received a similar dose of serum also proved to be sensitive when tested after three weeks.

This short protection does not seem very valuable, but it might be useful, under certain circumstances, to be able to guard against infection for a brief period, and we are satisfied that this can be effected with ease.

Durable Immunity. Serum and Virus by Simultaneous Side to Side Inoculation.

Mixtures of serum and virus injected together do not establish immunity, but it was clearly of great interest to see whether the method which has proved so successful in the case of Rinderpest and hog cholera would give good results in the case of dog distemper. We had a number of dogs available to give evidence on this point, for we had been testing the potency of serum by means of double, side to side, inoculation. All dogs which were treated in this way were later tested with fresh living virus, in large dosage, to see whether they were fully immune. They were tested at varying time intervals up to two months. Those animals which were tested about a fortnight after the double inoculation showed a temperature reaction when large doses of virus were used to test their immunity, but none of these animals died. When the test dose was not given until three weeks or a month after the double inoculation there was no reaction and no temperature disturbance, and there were no deaths. These results, though important, were not conclusive, for, without sufficient foresight, we had allowed the doubly inoculated animals to mix in one kennel with the control dog, and it might be argued with justice that the durable immunity was, in part, at any rate, due to the consolidating effect of repeated small doses of virus picked up from the control animal. On realising this possibility, we isolated the

serum and virus treated animals, and also performed a few experiments for the express purpose of testing the method. The results have been in every case highly satisfactory, and solid immunity to large doses of living virus has been demonstrated in every animal one month after the original inoculations.

It was desirable to find out if the dose of serum and virus had to be nicely balanced, or if excess of serum could be given without interfering with the development of immunity. For this purpose two puppies of one litter were taken, and one received a dose of serum known to be in excess of the amount required to eliminate all reaction to a small dose of virus, which was injected on the opposite side of the body. The second test animal received an equal dose of virus and four times the dose of serum given to the first animal. A control received virus only. After one month, when we know that any passive immunity attributable to the injected serum will have almost disappeared, a large test dose of virus was injected into each puppy. Neither animal showed any trace of reaction. It seems clear that it is allowable to give considerable excess of serum when immunising by this method without interfering with the development of immunity.

We were satisfied that this method might be made a practicable one for the immunisation of privately-owned hounds and dogs, and we have commenced to serve out limited numbers of doses to interested veterinarians.

This method of immunisation appears to us to offer certain advantages over the vaccine-virus method which we previously advocated, and, while not withdrawing anything we have said regarding the old method, we recognise certain ways in which the simultaneous inoculation method seems to be superior. First, we believe that side to side, simultaneous inoculation of serum and virus is often more convenient to the veterinary surgeon, as only one visit for inoculations is required. This may seem a small point, but it carries considerable weight with the practitioner and dog owner. Secondly, we believe that the method should prove on the whole more free from risks. In the case of the vaccine-virus method the dog had to play his part, and give a good response by producing sufficient antibody, after a short time interval, to prevent the virus causing serious trouble. In the method we are considering, prepared antibody is presented to the dog from the start. Thirdly, if, for any reason, there is a rise of temperature on the fourth or fifth day following inoculation, a dose of serum may be injected at once, and the threatened trouble suppressed. Finally, if it be desired to cut the risk down to the minimum, an excess of serum may be given, and, as we have seen, this does not appear to interfere with the development of solid immunity. There is no doubt that an available supply of antiserum—or, better still, concentrated antibody—gives a sense of security to the administrator of virus.

We must point out that though the method may be safer during

the inoculations it does not follow that the immunity will be so regular and so long lasting as in the case of vaccine followed by virus. It is quite possible that greater ease of mind as to the immediate result of the injections may be purchased at the cost of a less certain and a less durable immunity. Only time and a large number of inoculations can clear up this point.

We may say that so far the field inoculations have gone well, but, in spite of the fact that the dose of serum recommended was more than the calculated dose to give complete protection, there have been one or two cases of reaction following application of the new method. In every case the reaction has been controlled by the injection of extra serum, and there have been no fatalities. However, the number of animals treated is not very large (200), and we have not sufficient data to allow of any final judgment of the value of the method in the field, or adequate evidence as to the durability of the immunity produced by this technique.

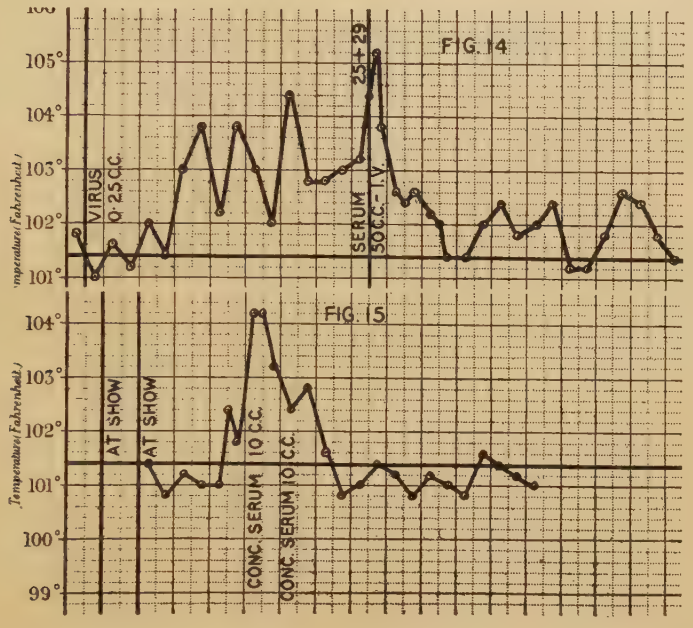
Treatment—Once distemper is established in the ferret we have found that massive doses of dog distemper antibody have no beneficial effect on the course of the disease. In the case of dogs, however, we have already shown in Section 1 that serum will produce an effect at any stage in the incubation period of distemper, and Figs. 4 and 8 show that a beneficial effect is still observable when the serum is administered at the time of the primary rise of temperature. We have also recorded the fact that in the few cases where a febrile reaction followed the double inoculation of serum and virus further doses of serum suppressed the threatened attack.

Fig. 14 illustrates the effect of a large dose of serum given late in the disease. The dog was initially a control animal for another experiment, and on the eighth day after inoculation he was acutely ill, becoming rapidly worse, and, in our opinion, his illness would probably have been fatal. 50 c.c. of a good hyperimmune serum was injected intravenously. There was an immediate serum reaction.* The lips and eyelids became very irritable, red, and within a few minutes œdematous. These symptoms passed off in about an hour, and by the following day the temperature was normal. The patient, though never showing pronounced fever again, remained weak, had persistent diarrhœa for ten days, and, in spite of taking food fairly well, lost condition. He ultimately made a perfect recovery.

Fig. 15 is a copy of the temperature chart of a Dandie Dinmont terrier, who apparently contracted distemper at one of two Shows which he attended, and at which he won prizes. The owner was seriously alarmed when a temperature of 104·2° F., developed on the fifth day after the first Show, and entrusted the dog to us in the hope that we might be able to cure him. On arrival, the dog was very dull and dejected, and the history, coupled with the typical temperature chart, made the diagnosis almost certain. However, 1 c.c. of

* Isolysins for dog red cells have been demonstrated in one batch of hyperimmune serum. The amount was small.

blood was taken and injected into a ferret to make quite sure of the diagnosis, and immediately thereafter 10 c.c. of concentrated antibody were injected subcutaneously. The following morning the dog was greatly improved and taking fluids freely. A second dose of



10 c.c. of purified antibody was given. The following day the temperature was normal, the dog fed freely and was quite alert. Uninterrupted recovery followed. The ferret developed distemper and was ultimately killed.

These experiments and a few similar ones have convinced us that potent serum and purified antibody are of great value in the treatment of early cases of distemper in the dog, and we have hopes that concentrated serum will prove to be of therapeutic value even in the later stages of the disease. Only time and considerable experience can elucidate this point.

Some calculations of potency are of considerable interest. It has been shown that 0·1 c.c. of a good serum will neutralise 10,000 infective doses of virus for ferrets, and thus 10 c.c. of serum will neutralise 1,000,000 infective doses of virus. A concentrated, purified antibody can be prepared containing at least five and probably twenty times this amount of protective doses, and we feel it would be very strange if a homologous product of this potency should prove to be without effect on the fully developed disease.

Calculations of protective units (if we may use such an expression) also throw interesting light on the condition of the dog which is resistant to infection. It has been shown that the small dose of 1 c.c.

of serum of good quality will render the recipient so far resistant that virus produces only a mild temperature disturbance. This means that antibody sufficient to neutralise 100,000 infective ferret doses, diluted and distributed throughout the fluids of the dog, are sufficient to render him nearly, if not quite, immune. A dog undergoing vaccination receives approximately 100,000 infective doses of virus killed with formaldehyde, and if these elicit the appropriate antibody the dog becomes resistant to infection. Viewed in this way, the success of the crude vaccine described by us in 1928 becomes more easily intelligible.

Summary and Conclusions.

1. Hyperimmune sera against dog distemper vary considerably in potency.

2. The order of merit of protective sera may be determined by experiments on dogs of known susceptibility.

3. The protective value of a serum is best estimated by determining what modification, if any, is induced in a distemper attack when small doses of serum are given in the incubation period of the disease.

4. Immune serum obtained from dogs will, under certain conditions, prevent distemper attacks in ferrets.

5. It has not yet proved feasible to standardise dog serum by the use of ferrets.

6. Hyperimmune serum, in addition to protective property, possesses the power of fixing complement in the presence of distemper virus.

7. Employing the serological reaction :—

(*a*) With a standard serum, a rough estimate of amounts of virus may be obtained.

(*b*) With a good virus, the potency of immune serum may be estimated.

8. The protective power of a serum and its complement fixing property have not yet been separated.

9. Hyperimmune serum may be fractionated and the protective power and the complement fixing property appear to be confined to one small fraction of the globulins.

10. Hyperimmune serum and purified antibody should be useful :—

(*a*) To confer immediate passive immunity, which, however, is of very short duration.

(*b*) To secure a durable immunity by simultaneous, side to side, inoculation of serum and virus ; and

(*c*) To treat distemper cases.

11. A method for the regular production of potent hyperimmune serum is described.

12. It should be possible to standardise distemper antisera by means of complement fixation tests coupled with experiments on compound-bred dogs.

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"STRUCK": ENTERITIS AND PERITONITIS OF SHEEP CAUSED BY A BACTERIAL TOXIN DERIVED FROM THE ALIMENTARY CANAL.

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The Romney Marsh, Kent, is remarkable for the good quality of its pastures and for the large number of sheep they support. The practice of grazing the pasture very heavily has prevailed for years, and it is not surprising that diseases, comparatively unknown in the surrounding country, should be all too familiar on the Romney Marsh. Locally these diseases are recognised as being of two distinct types, and are termed " gangrene " or " ganger " and " struck."

The terms " gangrene " and " ganger " are appropriate and refer to a wound gas gangrene, which may affect the ewe a day or two after lambing, or the lamb after castration or docking ; again, the disease occasionally appears in a flock of sheep soon after shearing. There is, however, always a clear history of a wound infection.

The term " struck " is applied to a disease characterised by a short period of illness which terminates fatally and is not associated with a wound infection.

Concerning the incidence of " struck " and the conditions under which it occurs, there is general agreement among owners and shepherds on the Marsh. The disease is said to occur during the late winter and spring, March being regarded as the time when it is most prevalent.

Certain pastures are notorious, while upon others the disease seldom occurs. The incidence of the disease seems to be intimately related to the amount of grazing available for the animals, and the losses from stock are believed to be heaviest when the pastures are short and the grazing is comparatively scanty, but in the absence of exact figures it is impossible to judge the toll exacted by this disease. Five to ten, or even fifteen, per cent. of the animals may be lost in one season on one particular portion of land, while on another the losses may be negligible.

The consensus of opinion is that " struck " is in some manner a dietetic disease in the sense that it is acquired through ingestion of certain pastures, but there is no suspicion that the disease is due to a plant poisoning, and from the fine quality of the pasture the incrimination of noxious weeds or plants seems out of the question.

The symptoms shown by the " struck " sheep are probably overlooked until grave lesions have developed, when the animals are noticed neither to feed nor to ruminate, and appear dull and dejected. Sheep may stand in a strained position, which has given rise to the erroneous opinion that the animals are straining to micturate. This position is probably assumed in an effort to relieve abdominal pain.

Diarrhœa is never alluded to in connection with " struck," nor is coughing, inco-ordination of movement, or cerebral symptoms.

The general knowledge regarding the post-mortem picture is very limited. The descriptions conveyed the impression that post-mortem appearances differed considerably, and that at times advanced changes were present in the organs of the abdominal cavity, while at others the musculature was affected and occasionally even the lungs. But it is common knowledge that if the sheep is detected before death and slaughtered the eviscerated carcase may be dressed for the butcher and sold for human consumption.

Generally no attempt is made to dispose of the carcases in a hygienic manner. The practice is for the shepherd to bring the carcase to a convenient point near his dwelling, skin it, leaving the skin out to dry, and feed the flesh to the dogs. Often no special effort is made to prevent sheep grazing on ground where carcases have lain, but there has been no suggestion that sheep on such ground were any more liable to contract the disease.

Twenty-five years ago Cave (1905) reported the conclusions arrived at after several years of study of the disease, and stated that " struck " and black-quarter were one and the same disease, caused by the same micro-organism, the black-quarter bacillus. Numbers and details of post-mortem and bacteriological examinations were not given in this communication nor in any of his Reports (Cave, 1903, 1904, 1907, 1909), and it is impossible to tell whether he ever worked with material obtained immediately after, or a short time after, the animal's death.

In the Report of the Departmental Committee on Louping-ill and Braxy (1906), the authors refer to the disease " struck " on the Romney Marsh, and say that : " the disease is caused by an anærobe closely allied to that of Braxy and Louping-ill and which, like them, is probably intestinal in habitat." Mention is also made of the view that " struck " is the same disease as black-quarter, but this view is not accepted, although no reasons are given for holding a different opinion other than the bald statement quoted above, that the bacillus is probably intestinal in habitat.

From enquiries made in 1928, it was at first thought probable that the diseases " struck " and " gangrene " were instances of gas gangrene infections and similar in nature, and arrangements were made to have specimens of what appeared to be affected muscles of animals which had died from these diseases forwarded to the Research Institute in Animal Pathology, Royal Veterinary College, London, where they would be examined for pathogenic bacteria.

These investigations proved of value and led to the isolation of strains of a new species of pathogenic sporulating anærobe, *B. paludis*, which appeared to play an important rôle in the ætiology of the disease " struck " (McEwen, 1930). This bacterium and the *V. septique* and *B. chauvœi* were the pathogenic bacteria frequently encountered in the investigations.

The results of the work conducted in London are given below in tabular form. In the Table 52 specimens are accounted for. This does not represent all the specimens received, which actually numbered 81. Of the 29 not included in the Table, three gave pathogenic anærobic bacteria, and one of these was probably *B. paludis*, but this strain was accidently thrown away before the typing was completed ; the other two were bacteria of the *B. œdematiens* type. In the case of the remaining 26 specimens failure in a number of instances to demonstrate pathogenic bacteria was experienced, but the majority were not examined as they were obviously in an advanced state of decomposition.

In many cases specimens were received with no history thereon ; these must be considered as coming from either a " struck " sheep or a sheep which had died of gas gangrene, but from which it is impossible to tell.

When more than one type of pathogenic anærobe were encountered in a specimen only that which appeared to predominate is included in the Table given below.

Summary of Results of Bacteriological Investigations Showing the Principal Types of Pathogenic Sporulating Anœrobic Bacteria Isolated from 52 Specimens Derived from Sheep said to have Died from " Struck," Post-parturient Gas Gangrene, or Wound Gas Gangrene.

	No. of specimens from which B. chauœvi *was isolated.*	*No. of specimens from which* V. septique *was isolated.*	*No. of specimens from which* B. paludis *was isolated.*
Specimens with a definite history of " struck "	0	2	6
Specimens from ewes with a history of post-parturient gas gangrene	2	3	0
Specimens from sheep with no definite history	10	18	6
Specimens from lambs with history of having recently been sheared or castrated ...	5	0	0

In a case of apparent *B. paludis* infection which is not included in the Table the history was one of " struck."

From many of these 52 specimens the particular bacterium isolated was present in large numbers to the exclusion of all other demonstrable ærobic or anærobic micro-organisms, and it was necessary when attempting to account for their presence to allow for their being ante-mortem invaders and possibly the cause of the animal's death. This is particularly true when the bacterium isolated is a comparatively fastidious micro-organism like *B. chauvœi*. It may not hold to the same degree for a vigorously spreading and easily recoverable micro-organism such as *V. septique*. In the particular cases from which *B. paludis* were isolated the striking picture presented by the specimens

made it impossible to think that this bacterium was a mere post-mortem invader and casually dismiss it as such.

1930 *Investigations on "Struck."*

The absence of reliable data on the pathology of the disease made it essential that cases should be investigated by post-mortem and bacteriological examinations. Post-mortem examinations have now been made at the time of the animal's death and up to 24 hours after death. A consideration of these examinations has shown that "struck" is a specific disease. But, as was to be expected, animals were sometimes said to be affected when they were suffering from or had died from a totally different disease.

When cultures were made from the tissues or body fluids these were invariably inoculated into broth containing minced meat and under a vaseline seal, and with rare exceptions the material was also inoculated into liver broth and on to liver agar or serum agar. These broth and agar tubes were incubated ærobically and anærobically. From the examination of the primary cultures information regarding the presence of one or more species of bacterium or the absence of bacteria from the seed material was obtained. Liberal quantities of seed material were used, and when body fluids were inoculated into a liquid medium quantities of 1 c.c. or more were sown into each tube.

Whenever *B. paludis* was recovered or was isolated from any particular source the identification of the bacterium in every instance is founded upon cultural characters, the production of *B. paludis* toxin by the particular strain in question, and the neutralisation of the toxin by specific antitoxic serum. The toxin neutralisation test was accepted as the real criterion of identification of the strain (McEwen, 1930).

The Macroscopic Lesions found in Cases of "Struck" where the post-mortem examination was made at the time of death and shortly after death.

Examinations of 17 cases have been made, and these showed remarkably constant lesions. These lesions are regarded as characteristic and consist of an enteritis, an acute peritonitis, and changes attributable to a toxæmia.

The peritoneal cavity contains an excessive quantity of fluid, varying in amount up to three litres or more. The fluid is pale yellow in colour, and lightly turbid, and a short time after death it becomes faintly blood-tinged. Yellow coagula floating in the fluid or adherent to the peritoneum are frequently found. Upon exposure to the air the fluid rapidly coagulates.

The peritoneal vessels are intensely injected and conspicuous, and multiple subperitoneal hæmorrhages may be present. Generally the injection of the vessels is most obvious over the omentum, the course of the small intestine, and the urinary bladder.

The mucous membrane of the abomasum occasionally shows

areas of mild congestion. The small intestine presents changes ranging from a moderate to a most acute congestion of part or parts of its length, and this is frequently accompanied by ulceration of the mucous membrane. The ulcers are generally present in the jejunum, and vary from small areas 2 or 3 mm. in diameter to extensive lesions 6 to 12 cm. in length by 1 to 2 cm. width. These ulcers may be numerous and involve many feet of bowel. The periphery of the ulcers is dark red and the floors are either deep red in colour or covered by a dark greenish adherent deposit. The ulcers rarely have been found extending down to the peritoneal covering of the intestine.

The contents of the small intestine are sometimes noticeably blood stained.

The cæcum and colon are frequently contracted, firm to the touch, with their lumen partially or almost completely obliterated and the mucous membrane thrown into multiple corrugations. When this condition is found fæcal material is absent or very scanty.

Individual mesenteric glands occasionally show a very moderate degree of congestion.

The kidneys sometimes appear to be in a state of cloudy swelling in cases examined at the time of death, the cut surface of the organ being irregular, glistening though dark in colour, and appearing to bulge outwards.

The urine and the urinary bladder have invariably been found normal. Hæmoglobinuria has never been observed.

On a few occasions congested areas have been found in the adrenals.

The liver and spleen appear healthy.

The thoracic cavity generally contains an excessive transudate of a clear yellow colour, and a similar fluid is often present in the pericardial sac.

Subepicardial and subendocardial hæmorrhages may be found in the heart.

The subcutaneous and intermuscular tissues are almost invariably normal, but a moderate infiltration of the abdominal intermuscular septæ with a pale semi-liquid material has been encountered in two instances. Lesions in the muscles suggestive of gas gangrene are not found.

Bacteriological Examination of 17 *Cases of "Struck."*

Material from 17 cases was examined by cultural and other means. In 13 instances *B. paludis* was isolated. In two cases *V. septique* was recovered, once along with *B. paludis* and once accompanied by non-pathogenic anærobic bacteria.

In the majority of cases where *B. paludis* was recovered the bacteria appeared to be widely distributed throughout the body, being recoverable from the heart blood and other organs. However, the bacteria were seldom numerous, except in cases where animals had been dead a few hours and microscopical examination of smears

of tissues and body fluids was frequently negative. Bacteria were most readily found in smears made from the peritoneal fluid. Bacteria have been recoverd from the peritoneal fluid when media seeded with heart blood and tissue from other organs has remained sterile. Failure to cultivate *B. paludis* from the body musculature has been experienced when the bacteria were recoverable from internal organs.

When *B. paludis* was isolated it was generally present in pure culture, but in one instance it was recovered from the peritoneal fluid along with *V. septique* though cultures from the thoracic fluid and spleen gave growths of *B. paludis* only. The only other case where *B. paludis* was not pure was when it was accompanied by a coliform bacterium in the peritoneal fluid.

From three most typical cases no bacteria were recovered. These cases were examined at the time of death, and in each instance the heart blood, peritoneal fluid, and spleen tissue were used for cultivation. In addition, in one instance the thoracic and pericardial fluids were similarly used, in another the thoracic fluid and material from a mesenteric lymph gland, and in the third case material from a mesenteric lymph gland.

B. paludis gives an abundant and vigorous growth, and had it been present there is little doubt but that it would have been demonstrated. The conclusion is therefore reached that *B. paludis* was not generalised throughout the bodies of these three sheep.

Ulcers were present, however, in the small intestine of these animals, and it is possible that bacteria associated with these ulcers by the production of toxin caused a toxæmia. But this explanation is not satisfactory because the disease may occur without there being ulceration, and in the absence of evidence of localised areas of bacterial activity the extensive lesions cannot be satisfactorily attributed to the scanty number of bacteria which may be found throughout the body.

The Histological Examinations of the Small Intestine in Cases of "Struck."

As the small intestine was the organ which showed the most definite inflammatory reaction, sections were prepared from parts macroscopically normal in appearance and from congested and ulcerated areas.

Material was obtained from five cases within a few minutes after death. This showed that the primary lesion consisted of a necrosis of the superficial parts of the mucous membrane. The necrosis progressively extends downwards to the submucous coat and is accompanied by a leucocytic infiltration of the still recognisable mucosa, together with congestion of the vessels and hæmorrhages therefrom.

In the early stage there is no evidence of bacteria in the mucosa, but as the extent of the necrosis increases bacteria may appear in the necrotic tissue, apparently invading it from the lumen of the bowel.

Sections of ulcers occasionally show masses of Gram-positive bacteria morphologically similar to *B. paludis* invading the deeper tissues at the periphery of the ulcer and giving rise to extensive collections of leucocytes in the invaded tissues. Ulcerated areas, however, may show no evidence of such active bacterial invasion of the tissues.

Post-mortem and Bacteriological Examinations Conducted more than eight hours after death.

A number of the cases examined more than eight hours after death were in a very advanced state of post-mortem decomposition and no reliance could be placed on data obtained from them. These cases, therefore, were not examined bacteriologically, and they are not considered here. However, in 13 examinations conducted eight or more hours after death post-mortem changes were less advanced and bacteriological examinations were made.

The most striking changes found were in the subcutaneous, intermuscular, and muscular tissues. The subcutaneous and intermuscular areolar tissues showed various degrees of infiltration with a red, semi-fluid, gelatinous exudate, while the muscles themselves were moist, extremely soft and frequently discoloured in patches, the colour varying from a dirty salmon-pink to a reddish-black. In some instances, the muscles were emphysematous and riddled by gas. The whole picture was very suggestive of acute gas gangrene infection, and the gross appearance comparable with black-quarter.

Another notable feature was the large accumulations of turbid, blood-stained fluid in the abdominal cavity. A similar fluid was sometimes present in the thoracic cavity. In some instances inflammatory reactions were detectable in the alimentary tract, and ulceration of the small intestine has been observed.

Of the 13 cases included in this group, 11 showed the characteristic changes reminiscent of gas gangrene in the subcutaneous areolar tissues and muscles, in nine cases there was excess of fluid in the peritoneal cavity, in four instances some excess of fluid was found in the thoracic cavity, and in four cases evidence of inflammatory changes in the intestines was found.

Smears made from the abdominal fluid showed that more than one species of bacterium was present. However, smears made from the musculature revealed in ten cases a bacillus, morphologically similar to *B. paludis*, in great abundance and apparently uncontaminated. In another case the same bacillus was present in a state of purity in the smears made from the spleen. From seven of these cases *B. paludis* was recovered in pure culture, and from one case *V. septique* was also isolated, although it could only have been present in the tissues in small numbers, and its presence was not suspected by smear examination. In the remaining five cases in which bacilli resembling *B. paludis* were found in smears difficulty was encountered in obtaining pure cultures, and the information obtainable from these did not seem to justify the time which would

have been necessary to purify them. From one of these mixed cultures *V. septique* was readily obtained in a pure state.

Smears from the remaining two cases showed moderately large bacteria, not of the *B. paludis* type. From one of these *V. septique* was isolated, and from the other an unknown anærobic bacterium of low pathogenicity.

From the bacteriological examinations it appeared that *B. paludis* was the bacterium principally involved and responsible for the extensive changes encountered in the subcutaneous, intermuscular, and muscular tissues. The outstanding feature of the post-mortem examinations was the changes in these tissues, and it was at first difficult to reconcile them with the absence of lesions in the muscles and subcutaneous tissues at or soon after death ; but, as *B. paludis* was the micro-organism which could be held responsible in the majority of cases, there was little reason to doubt that the changes which had formerly given rise to the conception that this disease was analogous to black-quarter were of post-mortem generation. The possibility of this was proved by experiment.

The Production of the Changes in the Musculature which Simulate Black-quarter.

The peritoneal fluid of a field case of " struck " (Sheep W. 42) which was obtained six hours after death contained very large numbers of *B. paludis* and was shown to be rich in *B. paludis* toxin.

1.—Approximately 150 c.c. of the unfiltered peritoneal fluid from Sheep W. 42 were inoculated intravenously into a normal sheep. The animal collapsed immediately the inoculation was made, and was dead within six minutes. The post-mortem examination was purposely delayed until 15 hours later, the carcase being allowed to lie in the field, the weather at the time being cool. The subcutaneous tissues contained an abundant accumulation of dark red, soft, gelatinous material and similarly tinted fluid. This was most plentiful in the regions of the neck, axillæ, and back. Similar infiltrations were present in the intermuscular septa. The muscles had a dirty salmon-pink colour, with here and there parts of a deeper and darker tint ; they were moist, very soft and friable, and in places riddled with gas. The subcutaneous and muscular tissues presented a picture exactly comparable to that so frequently met with in cases in the field when the post-mortem examination was made more than eight hours after death. The remaining organs showed putrefactive changes only. No specific lesions were noted in the abomasum or intestines, and no excess of fluid was present in the abdominal cavity.

Smears made from the muscle showed a very rich and apparently pure culture of a bacillus morphologically similar to *B. paludis*, and this bacillus was recovered in cultures.

2.—Approximately 180 c.c. of bacteria-free filtrate of the peritoneal fluid from Sheep W. 42, diluted with an equal volume of saline, were inoculated intravenously into a normal sheep. The animal collapsed

while the material was yet being inoculated and after a few convulsive struggles it died. Death occurred about five minutes after the inoculation had been made. This sheep had been inoculated within a few minutes of the inoculation of the previous animal, and again the carcase was left lying in the field and the post-mortem examination made the following morning, approximately 16 hours after death. The subcutaneous tissues contained a small quantity of gelatinous material. The muscles were moist, but neither soft nor spongy, nor riddled with gas. The difference in the appearance of the subcutaneous, intermuscular, and muscular tissues of this sheep and the corresponding tissues of the sheep inoculated with the unfiltered peritoneal fluid which contained *B. paludis* was most striking. In the present case there was no evidence of changes simulating the appearance of black-quarter or gas gangrene. In other respects, the post-mortem examination resembled that of the previous sheep.

Smears were made from the muscle tissues and these showed a few very large bacteria but they did not resemble *B. paludis*, and in cultures made from the muscles there was no evidence of the presence of that organism.

There is no doubt that the changes found in the muscular, intermuscular, and subcutaneous tissues of the animal which was inoculated with material containing bacteria were produced by the post-mortem generation of those bacteria.

It is only in the " struck " sheep which has been dead some considerable time that changes simulating black-quarter are found. These, being post-mortem changes, dismiss the suggestion that " struck " and black-quarter are similar diseases.

The Experimental Production of " Struck."

The occasional inability to demonstrate pathogenic bacteria in body fluids and tissues of sheep which have died from " struck," and the improbability that the ante-mortem invasion of the body with pathogenic bacteria was responsible for the lesions encountered at or soon after death, led to the experimental inoculation of healthy sheep with the body fluids and tissues of " struck " sheep collected at the time of death.

1.—Portions of three congested mesenteric lymph glands from Sheep W. 26 (tissues sterile), together equivalent to the size of a walnut, were ground up in a mortar with sterile saline, and the emulsion obtained was inoculated intravenously into a sheep. The animal remained well.

2.—Six grams of spleen from Sheep W. 26 were ground up in a mortar with sand and 40 c.c. of saline. Twenty c.c. of this blood-stained suspension of the spleen tissue was inoculated intravenously into a sheep. The animal remained well.

3.—A congested mesenteric lymph gland from Sheep W. 58 (tissues sterile) was ground up in a mortar with 20 c.c. of saline, and the whole was inoculated intravenously into a sheep. The animal remained well.

4.—A congested mesenteric lymph gland from Sheep W. 58 was ground up in a mortar with 20 c.c. of saline, and the whole inoculated subcutaneously

into a sheep. At the seat of inoculation a local reaction occurred, but otherwise the animal remained normal.

5.—The peritoneal fluid from Sheep 26 contained no demonstrable toxin when 6 c.c. were inoculated intravenously into rabbits. Twenty c.c. of the peritoneal fluid from Sheep W. 26 was inoculated into a sheep. The animal remained well.

6.—Ten c.c. of the peritoneal fluid from Sheep W. 57 (*B. paludis* generalised in body) were centrifuged and inoculated intravenously into a rabbit, but no evidence of toxin was obtained.

Two hundred c.c. of the peritoneal fluid from Sheep W. 57, after filtration through a Seitz filter, were inoculated subcutaneously into a sheep. The animal remained well.

7.—Two hundred c.c. of the unfiltered peritoneal fluid from Sheep W. 57, shown by cultural methods to contain *B. paludis*, were inoculated intravenously into a normal sheep. The animal remained well.

8.—Ten c.c. of the peritoneal fluid from Sheep W. 58 were inoculated intravenously into a rabbit, and no toxin was demonstrated.

One hundred and twenty c.c. of the peritoneal fluid from the same sheep were inoculated intraperitoneally into a sheep. The animal remained well.

Summary of the Experimental Inoculation of Normal Sheep with Material Obtained from Sheep Affected with "Struck."

The intravenous and subcutaneous inoculation of sheep with saline emulsions of mesenteric lymph glands and the intravenous inoculation of sheep with emulsion of spleen tissue which have contained no demonstrable bacteria and were derived from sheep which had died or had been killed while suffering from "struck," did not result in the transmission of the disease or in the production of any signs of illness.

The peritoneal fluid from affected sheep used in these transmission experiments was tested for toxin by inoculating moderate quantities of the fluid into rabbits by the intravenous route. No toxin was demonstrated, and the intravenous inoculation of large quantities of the peritoneal fluid into sheep, even though it contained *B. paludis*, as was the case of that derived from Sheep W. 57, was without harmful effect. Further, the intraperitoneal inoculation of peritoneal fluid and the subcutaneous inoculation of filtered peritoneal fluid produced no effect on the experimental animals. The failure to produce the disease in any of the above experiments makes it most unlikely that the disease is an inoculable one caused by a virus present in the affected lymph glands, the spleen, or the characteristic exudates in the peritoneal cavity.

The Oral Administration to Normal Sheep of Body Fluid and Tissue and the Contents of the Alimentary Tract of Animals which had died from "Struck."

1.—The intestinal contents from Sheep W. 26 were collected, and approximately 900 c.c. of these were fed to a sheep. The animal remained well.

2.—Twelve feet of the small intestine, containing ulcerated areas, were chopped into small pieces one-half to two centimeters in length, and fed to a sheep. The animal remained well.

3.—Some of the peritoneal fluid of Sheep W. 42 which contained large numbers of *B. paludis* was mixed with an equal volume of normal saline solution and filtered through a Seitz filter. One c.c. of the filtrate was shown to contain sufficient *B. paludis* toxin to kill a rabbit in a few minutes.

Six hundred c.c. of this peritoneal fluid, unfiltered, and containing *B. paludis*, were fed to a sheep. Fifteen hours later the animal was found dead. Rigor mortis was present, and blood-stained froth and liquid were present at and around the nostrils. The mucous membrane of the conjunctiva was congested. Small areas of gelatinous infiltration were found under the skin of the neck. The muscles were moist, but showed no other abnormalities. The peritoneal cavity contained over one litre of turbid, blood-tinged fluid, and all the organs in this cavity were in a moderately advanced state of putrefaction. The kidneys and liver were soft and foamy, and the spleen was slightly swollen and soft. An excessive quantity of fluid was present in the thoracic cavity. The lungs were soft and decomposed, the apical lobe of each lung being chiefly affected. Doubt was entertained as to whether some of the peritoneal fluid had entered the lungs when the animal was fed and had accelerated the sheep's death, and after this the stomach tube was used in feeding experiments. However, the excess of fluid in the peritoneal cavity encouraged the suspicion that the cause of the animal's death was similar to that which brought about such extensive exudations into the peritoneal cavity of sheep dying from " struck " in the field.

Smears made from the peritoneal fluid of this experimental sheep showed a rich mixture of bacilli, but smears made from the spleen pulp showed large numbers of bacilli morphologically similar to *B. paludis* and a few longer and more slender bacilli ; smears made from the muscle tissue showed an apparently pure culture of bacilli morphologically similar to *B. paludis*.

Cultures made from the spleen and the muscle in both instances gave bacilli and colonies identical with *B. paludis* and its colonies, and the inoculation of a guinea-pig with cultures produced typical *B. paludis* lesions (McEwen, 1930).

Six hundred c.c. of the peritoneal fluid from the experimental sheep were fed to a sheep. The animal remained well.

The contents of the abomasum and the small intestine of the experimental sheep were fed to a sheep. The animal remained well.

4.—The peritoneal fluid of Sheep W. 57 and W. 58 were both devoid of demonstrable *B. paludis* toxin, although cultural tests showed that *B. paludis* was present in the peritoneal fluid of Sheep W. 57. The peritoneal fluids from these two animals were mixed in equal proportions, and 2,500 c.c. of the mixture were given by the mouth to a sheep. The animal remained well.

5.—The contents of the abomasum and the small intestine of Sheep W. 58, amounting in all to 1,500 c.c. of material, were similarly given to a sheep. The animal remained well.

Summary of the Experimental Feeding of Normal Sheep with the Body Fluids and Tissues and the Contents of the Alimentary Tract of Animals which had died from " Struck."

Experiment No. 3 gave encouraging results in spite of the fact that its value may be somewhat discounted by the possibility that some of the material which was given entered the lungs though no conclusive evidence of this was obtainable. From this experiment it would seem that if peritoneal fluid which contains large numbers

of *B. paludis* and its toxin is administered to a healthy sheep, it may bring about death in a matter of a few hours, producing exudations of fluid into the peritoneal cavity not dissimilar to those met with in natural cases.

The oral administration to normal sheep of large quantities of peritoneal fluid derived from an experimental sheep and containing large numbers of bacteria, but not large numbers of *B. paludis*, failed to produce any symptoms, and the administration of a very large quantity of peritoneal fluid from natural cases when the fluid contained no demonstrable toxin and only a very few *B. paludis*, failed to produce any symptoms in a healthy sheep.

The disease was not produced by feeding intestinal and abomasal contents or by feeding the ulcerated small intestine to sheep.

The evidence obtained from the experiments, therefore, would indicate the possibility of producing the disease experimentally by feeding material rich in *B. paludis* and its toxin.

The Oral Administration of Toxic Filtrates of B. paludis *to Sheep.*

The filtrates used in the experiments were obtained from 18 to 24-hour growths in glucose liver broth. All the feeding experiments were conducted with the stomach tube.

1.—Four litres of glucose liver broth exactly comparable to that used for the growth of cultures, but with the *p*H adjusted to 4·6, were given to a sheep. The animal was in no wise upset, and remained healthy.

2.—The filtrate from a culture of *B. paludis* was inoculated intravenously into a rabbit in an amount of 0·5 c.c. This killed the rabbit in six minutes.

One thousand eight hundred c.c. of the filtrate were then given by the stomach tube to a sheep. The animal in all respects remained well.

3.—A culture of *B. paludis* was filtered. The filtrate in 1 c.c. amount was inoculated intravenously into a rabbit. This killed the animal in twenty minutes, and 2 c.c. inoculated intravenously killed a rabbit in eight minutes.

Four thousand five hundred c.c. of this toxic filtrate were given by the stomach tube to a sheep. The animal in all respects remained normal and well.

4.—0·5 c.c. of a *B. paludis* filtrate, when inoculated intravenously into each of two rabbits, produced in ten minutes symptoms of profound collapse and weakness, and death two hours after the inoculation.

Five thousand seven hundred c.c. of this filtrate were given by the stomach tube to a sheep. The animal was not allowed to feed until three hours after the experiment. It was then turned into a field, and immediately it commenced to graze. At the end of the fifth hour it was killed for the purpose of searching the alimentary tract for evidence of toxin. No lesions were found.

The ingesta in the different portions of the alimentary canal were collected and measured. The rumen and reticulum contained 10,000 c.c., the abomasum 350 c.c., the first part of the small intestine 260 c.c., the middle part 700 c.c., and the last part of the small intestine 800 c.c. ; the large intestine 1,300 c.c. of material.

The total amount of material in the alimentary tract was approximately 13,400 c.c., and a considerable portion of this must have come from the 5,700 c.c. of filtrate fed to the animal.

From the more than usually liquid character and the large volume of fluid in the rumen and reticulum it appeared as though most of the filtrate

which had been fed was still present in these chambers. The ingesta in the abomasum and first two-thirds of the small intestine was also liquid in character, that in the terminal third of the small intestine was less so, while the contents of the large intestine were moist but not liquid in consistency.

If ingestion, absorption, and defæcation during the five hours preceding death are disregarded, it may be assumed that of the 13,400 c.c. collected at death approximately 7,700 were present in the alimentary tract before feeding the filtrate, and, assuming that the filtrate had been equally mixed throughout the alimentary tract, then every 1·17 c.c. of ingesta should contain an amount of toxin equivalent to that in 0·5 c.c. of the original filtrate, but with the evidence pointing to richer admixture of the filtrate in the rumen and reticulum it is reasonable to suppose that there was a higher concentration of filtrate per cubic centimeter in their contents.

The contents of the different portions of the alimentary tract were centrifuged, and the supernatant fluids inoculated into rabbits with a view to ascertaining whether toxin was present in a concentration which might reasonably be expected, had destruction or alteration of the toxin not already occurred.

Ten c.c. of fluid collected from the contents of the rumen and reticulum were inoculated intravenously into a rabbit. The animal showed no symptoms, and the following day it was well.

Ten c.c. of fluid from the contents of the first portion of the small intestine were inoculated intravenously into a rabbit. Three minutes after the inoculation the animal showed symptoms of weakness and dyspnœa, but in half an hour these had passed off, and the animal was well on the following day.

Ten c.c. of fluid from the middle portion of the small intestine were inoculated intravenously into a rabbit, with a result identical to that of the preceding case.

Even after prolonged centrifuging it was impossible to obtain more than a few cubic centimeters of a turbid fluid from the cream-like contents of the terminal portion of the small intestine. Five c.c. of this fluid inoculated intravenously into a rabbit killed the animal in four minutes. Only six more cubic centimeters of this fluid were available. Three of these were mixed with 2 c.c. of *B. paludis* antiserum, and 3 c.c. were mixed with 2 c.c. of *B. welchii* antiserum. These mixtures, after standing at room temperature for one hour, were inoculated intravenously respectively into each of two rabbits. In each instance the rabbit showed symptoms of weakness a few minutes after the inoculation, but recovered within forty minutes It was therefore concluded that the toxic element in the contents of the small intestine was not *B. paludis* toxin, that none of this toxin was demonstrable, and that in the course of five hours it had been largely, if not completely, destroyed or altered in the alimentary tract.

From these experiments it is assumed that the administration of large quantities of nutrient broth and of filtrates of *B. paludis* containing toxin was harmless for sheep and that the toxin was rapidly altered or destroyed in the alimentary canal, and further, that destruction of toxin occurred in the rumen and reticulum, which makes it improbable that anything but a small amount of unaltered toxin reaches the abomasum when the toxin has been fed.

The Oral Administration of Cultures of B. paludis.

The cultures used were in all instances twenty to twenty-four hour cultures in liver broth, and had a *p*H of 4·5 to 4·6.

The amounts of culture which were given were very large and the numbers of bacteria greatly in excess of what could be ingested naturally by animals, but in any feeding experiment it must be remembered that the great mass of ingesta passes directly into the rumen and reticulum, in which receptacles it becomes diluted with the semi-liquid food there present and only gradually and slowly does it find its way to the true digestive and absorptive portions of the alimentary tract. Further, the cultures consisted entirely of vegetative bacteria and it is possible that many of these are destroyed before reaching the abomasum and small intestine, and, judging from the appearance of smears made from the different portions of the alimentary tract of animals which have been given cultures, this would appear to be the case.

Up to the present, it has not been found possible to obtain cultures of sporulating bacilli suitable for feeding experiments.

In the experiments recorded below large amounts of culture were fed in the hope that if excessive qualities of fluid reached the rumen and reticulum some, at least, would rapidly find its way into the œsophageal groove and reach the abomasum.

1.—The indication that " struck " might be produced by feeding peritoneal fluid which contained large numbers of *B. paludis* led to an attempt to produce the disease by feeding non-toxic peritoneal fluid mixed with a broth culture of the bacterium.

A sheep was fed at hourly intervals with this mixture until six doses had been given, equivalent to 190 c.c. of culture and 120 c.c. of peritoneal fluid. A final dose of 250 c.c. of culture alone was then given. The animal remained well.

2.—One litre of a broth culture was fed to a sheep. The animal remained well.

3.—Two litres of broth culture were fed to a sheep. The animal ceased to feed, diarrhœa ensued, and loss of condition was rapid ; the animal became very weak, and at the end of a week it was not expected to live. After ten days, however, an improvement set in, and during the next week the appetite became normal, and there was a decided improvement in condition. The animal was slaughtered sixteen days after the date of the experimental feeding. The carcase was in a poor condition. The only discoverable lesion was a collection of a yellowish-brown gelatinous material between the left peritoneal surface of the rumen and the abdominal wall. The gelatinous material was traversed by fibrinous strands, some of which were adherent to the rumen and the abdominal wall, while others had free ends extending several inches beyond the gelatinous mass. The remainder of the post-mortem examination was of an entirely negative character.

4.—Four litres of culture were fed to a sheep at 3.30 p.m. The same evening the animal was observed to be lying down more than normally. It was neither grazing nor ruminating, but apart from these signs it was apparently bright and active. The following morning the animal was dull, and appetite and rumination were still in abeyance. By mid-day no further

change was noticeable, and the animal when driven appeared like a healthy sheep. By 7 p.m., a distinct change for the worse was evident, and the animal was standing with its head hanging down and ears drooping, and some time during the night, approximately 36 hours after it had received the dose of culture, it died. When it was found at 8 a.m. in the morning rigor mortis was present and the cornea was opaque. The peripheral parts of the body were cold, but the inguinal regions were still warm to the touch. The peritoneal cavity contained 1,500 c.c. of a yellow and slightly turbid fluid which coagulated on exposure to the air. The peritoneal vessels were intensely injected, and small hæmorrhages were present under the peritoneum. The mucosa of the abomasum in the region of the pylorus was congested, and here and there small hæmorrhagic areas were present in it. The interior of the first 25 ft. of the small intestine showed a number of small areas of congestion, and all of these were deeply injected and suggestive of a stage preceding that of ulceration. The following 15 ft. of the small intestine were acutely congested throughout and the contents were liquid and blood-stained. The remainder of the small intestine showed areas of congestion particularly well defined at the Peyer's patch. The extremity of the cæcum was contracted and the mucous membrane thrown into ridges, and here and there the mucous membrane of this organ appeared to be congested. The colon was contracted, and areas of its mucous membrane were moderately congested. A small quantity of fluid was present in the thoracic cavity. Over the heart were scattered a few subepicardial punctiform hæmorrhages. Under the endocardium of the left ventricle there were numerous hæmorrhagic areas.

Smears made from the peritoneal fluid showed a number of bacilli of *B. paludis* type, five to ten bacteria per field. Smears made from the heart blood, the intestinal lymphatic glands, and the spleen showed no micro-organisms.

Media inoculated with spleen pulp failed to show any sign of bacterial growth, and no ærobic bacteria were recovered from the heart blood and an intestinal lymph gland, but from the heart blood and peritoneal fluid bacilli of *B. paludis* type were recovered in the cultures incubated anærobically.

This case in all its features was identical with a field case of " struck."

5.—Six litres of *B. paludis* culture were given by stomach tube to a sheep at 10 o'clock in the morning. By the afternoon the animal appeared to be a little dull and was not observed to feed. By the evening it showed symptoms of uneasiness, particularly a tendency to stand with its hind legs stretched out in a strained position.

On the following morning, at 7 a.m., the sheep was found dead. The surface of the body was quite cold, the cornea was opaque, and rigor mortis appeared to be passing off. From these indications it was probable that the sheep died in the early hours of the morning, 14 to 16 hours after the administration of the culture. The post-mortem examination revealed a typical case of " struck."

Smears made from the abdominal fluid showed pure cultures of bacteria morphologically similar to *B. paludis*.

The peritoneal fluid, heart blood, and spleen pulp were cultivated, and in each case a bacillus of *B. paludis* type was the only micro-organism recovered. The peritoneal fluid strain was identified as *B. paludis*.

6.—A sheep which had been fed in the stall upon a ration of clover hay and three-quarters of a lb. of linseed cake per day was fed with two litres of

broth culture and one litre of nutrient broth. The animal remained well. It was removed to a small paddock where the grazing was very scanty and a small supplementary ration of oats was given. Two weeks later it was fed with 4½ litres of broth culture. Twenty-four hours after it had been fed the animal was found in a collapsed condition and chloroformed. The post-mortem examination revealed the characteristic picture of " struck."

Smears made from the deposit obtained by centrifuging 50 c.c. of citrated peritoneal fluid showed no bacteria, but masses of leucocytes and an occasional epithelial cell. Smears made from the material in the different portions of the alimentary tract showed bacilli of *B. paludis* type in the abomasum and small intestine only. In the small intestine these bacilli were very numerous.

The heart blood and peritoneal fluid were cultivated. No growth was obtained from the former, but a bacillus morphologically similar to *B. paludis* was obtained from the peritoneal fluid.

Summary of and Discussion on the Experimental Feeding of Sheep with Cultures of B. paludis.

Two sheep, grazing in pasture, were fed by stomach tube with large quantities of culture, four to six litres respectively. A few hours after feeding signs of illness were seen. The animal fed with four litres died within 36 hours. In the case of the animal fed with six litres the illness was more acute and death followed in from 14 to 16 hours.

The post-mortem examinations made on these two sheep revealed lesions which were characteristic of the disease " struck," and the bacteriological examinations of the body fluids and tissues corresponded with those made from natural cases of the disease a few hours after death. In these two experimental sheep ulceration of the small intestine was not present, but this is frequently absent in field cases of " struck."

One sheep fed in the stall was not affected by feeding two litres of culture and one litre of broth, but later this sheep when fed with 4½ litres of culture became affected with typical " struck."

One sheep fed with two litres of culture was rendered seriously ill but eventually recovered.

It is, therefore, considered that the disease has been experimentally produced by administering culture of *B. paludis* by the mouth, but again, as exemplified by the case of the experimental sheep which was killed 24 hours after the administration of culture, the nature and extent of the lesions could not be attributed to the very scanty invasion of the body by bacteria.

In view of the findings obtained, it would appear that the disease might be attributed to the production of toxin by *B. paludis* in the absorptive portions of the alimentary tract, the toxin causing necrosis of the mucous membrane of the intestine and passing into the body giving rise to toxæmia.

The fact that the administration of toxin by the mouth did no produce disease may be attributed to a destruction of the toxin, either before it reaches the masum and small intestine, or to a rapid destruction and adsorption in e lumen of the abomasum and s intestine of any toxin which escaped from the rumen and reticulum.

Were the disease to be produced by feeding bacteria without their toxin it would constitute further proof of toxin production in the alimentary canal from whence the toxin reached the body.

The Oral Administration of B. paludis *collected by Centrifuging Broth Cultures, the Bacteria being re-suspended in Fresh Nutrient Broth.*

Fresh nutrient broth was used in the preparation of bacterial suspensions in preference to normal saline.

The animals used had previously been given cultures of *B. paludis* by the mouth whilst they were living in a stable and subsisting on a ration of maize, mangels, and hay. These animals had, contrary to what had been expected, remained well. For the two months prior to the present experiment they had been grazing on permanent pasture.

Two sheep were both fed with bacteria collected by centrifuging 6 litres of broth culture of *B. paludis*. The bacteria were suspended in 5 litres of liver broth and 2 litres of meat infusion broth; both broths contained 0·4 per cent. glucose.

One sheep, No. 18, was fed with $2\frac{1}{3}$ litres, and the other sheep, No. 19, with $4\frac{2}{3}$ litres. Twelve hours after the material had been fed, the sheep appeared to be well. No. 18, however, was chloroformed, and a post-mortem examination was made. No macroscopic changes were found in any of the organs.

Histological examination of different portions of the small intestine showed in each instance a necrosis of the lining epithelium and a destruction of the epithelium of the more superficial intestinal glands, together with an extensive leucocytic infiltration of the mucous membrane. This infiltration was most extensive in the superficial third of the mucous coat. Eosinophiles were numerous among the infiltrating leucocytes. There were no bacteria in the tissues.

The destruction of the epithelium and the extensive leucocytic infiltration in the mucosa are quite in keeping with the histological picture obtained in cases from the field; in the latter the process had extended further, and necrosis was more advanced.

The sheep fed with the bacteria suspended in $4\frac{2}{3}$ litres of broth was obviously very ill and unable to move 36 hours after it had been fed. It was, therefore, chloroformed, and a post-mortem examination made; this was in all respects typical of the disease. In the jejunum there were dark red areas up to 1 cm. in diameter, which suggested the incipient stage of ulceration.

Sections made from the areas of incipient ulceration in the small intestine showed a complete destruction of the mucous membrane down to the submucous coat, the mucous membrane being replaced by an amorphous mass of material detached from the submucosa. Throughout this material and invading the submucous tissues there were considerable numbers of Gram-positive bacilli morphologically similar to *B. paludis*.

In sections made from portions of the intestine which macroscopically appeared normal there was a destruction and necrosis of the superficial third of the mucous coat, together with an extensive leucocytic infiltration throughout the remainder of the mucous membrane, but there was no evidence of any bacterial invasion of the tissues.

Smears made from the peritoneal fluid showed no bacteria, and media inoculated with peritoneal fluid remained sterile.

3.—Three and a half litres of broth culture were fed to a sheep.

4.—The bacteria collected by centrifuging 3½ litres of broth culture were suspended in the same quantity of nutrient broth and fed to a sheep.

Sixteen hours after feeding the animals were ill and disinclined to move, and when made to do so moved in a tucked-up and painful manner, the movements of the limbs being short and stiff. Signs of illness being unmistakable both sheep were chloroformed and post-mortem examinations made.

At the post-mortem the sheep fed with the broth culture presented the characteristic changes associated with "struck." No micro-organisms were found in smears made from the peritoneal fluid, but a bacillus of the *B. paludis* was obtained in cultures therefrom. No other bacteria were present.

Smears made from the contents of the different portions of the alimentary tract indicated that the only part where bacilli morphologically similar to *B. paludis* were present in any number was the abomasum.

The post-mortem examination of the animal fed with the bacteria suspended in nutrient broth showed typical but not advanced lesions. Smears made from the peritoneal fluid showed no micro-organisms, but in cultures a bacillus of *B. paludis* type was obtained. No other bacteria were present.

Smears made from the food material from different portions of the alimentary tract showed numbers of bacilli morphologically resembling *B. paludis* in the deuodenal contents only.

These experiments show that the disease may be produced by feeding bacteria without toxin.

Again, it is not considered probable that in those cases the enteritis, peritonitis, and effusions of fluid in the serous cavities could be attributed to bacterial multiplication in the body.

The only definite foci of bacterial multiplication which have been found in natural and experimental cases have been confined to well defined localised areas of extensive destruction and ulceration of the mucous membrane of the intestine. If bacteria multiplying in the body tissues were the cause of the disease it would be reasonable to assume that the more extensive the ulceration the more acute would be the disease. But this has not been the case in experimental animals, indeed the two which showed the longest survival period were the only ones in which deep localised areas of necrosis of the mucous membrane were found, and in natural cases ulceration has been more common in cases where there was reason to believe that the disease ran a less rapid course. Indirect evidence would, therefore, rule out the bacteria associated with the ulcers as the primary cause of the disease.

B. paludis *Toxin in the Contents of the Alimentary Canal in Field Cases of "Struck."*

Should it be possible to demonstrate *B. paludis* toxin in the contents of the alimentary canal very important evidence would be obtained in support of the theory that the disease is due to the passage of toxin from the alimentary canal into the body.

The rapidity with which a sufficiently large dose of *B. paludis* toxin causes death in the intravenously inoculated rabbit was particularly helpful in these experiments because it enabled the presence, absence, or neutralisation of *B. paludis* toxin in the material tested to be decided

TABLE SHOWING THE RESULTS OF THE SEARCH FOR *B. paludis* TOXIN IN THE PERITONEAL FLUID AND THE CONTENTS OF THE ABOMASUM AND SMALL INTESTINE IN NATURAL CASES OF "STRUCK." INOCULATIONS WERE INTRAVENOUS, AND RABBITS WERE USED.

Sheep from which material was obtained.	*Interval between death and the collection of the material.*	*Material from.*	*Amt. inoculated.*		*Serum*			*Result of inoculation*	*Remarks.*
			Supernatant fluid.	*Filtrate*	*Normal Rabbit.*	B. paludis.	B. welchii.		
W. 49	40 minutes		5 c.c.						*B. paludis* toxin was found in the contents of the small intestine and in considerable concentrations; 5 c.c. of the material containing the filtrate from 2·5 c.c. of intestinal contents killed rabbits in a few minutes.
,,	,,	Abomasum diluted with an equal volume of saline		5 c.c.				None	
,,	,,	Small intestine diluted with an equal volume of saline		6 c.c.				Dead in 6 minutes; typical *B. paludis* intoxication	
,,	,,	,,		6 c.c.	2 c.c.			Dead in 10 minutes	
,,	,,	,,		6 c.c.		2 c.c.		None	
,,	,,	,,		6 c.c.			2 c.c.	Dead in 7 minutes	
,,	,,	(Another lot of filtrate)		5 c.c.	2 c.c.			Dead in 7 minutes	
,,	,,	,,		5 c.c.		2 c.c.		None	
W. 53	15 minutes	Abomasum		5 c.c.				None	No evidence of *B. paludis* toxin
,,	,,			10 c.c.				None	
,,	,,	First half of small intestine		5 c.c.				None	
,,	,,	Latter half of small intestine		5 c.c.				None	

W. 54	A few minutes	Abomasum	5 c.c.				Dead in 2 minutes	
,,	,,	,,	5 c.c.		2 c.c.		Dead in 2½ minutes	
,,	,,	,,	5 c.c.			2 c.c.	None	*B. paludis* toxin was found in the contents of the abomasum, but it was not demonstrated in the comparatively small quantities of material from the small intestine which were tested
,,	,,	,,	3 c.c.				Weakness followed inoculation, died during the night	
,,	,,	First third of small intestine diluted with third of its volume of saline		3 c.c.			None	
,,	,,	,,	3 c.c.				None	
,,	,,	Middle third of small intestine		5 c.c.			None	
,,	,,	,,		4·5 c.c.			None	
W. 57	A few minutes							
,,	,,	Abomasum		5 c.c.				A toxin was present in the contents of the small intestine but in low concentration, and toxin neutralisation tests were not made
,,	,,	,,		10 c.c.			None	
,,	,,	Small intestine plus an equal volume of saline		4 c.c.			None	
,,	,,	,,		8 c.c.			One minute after inoculation weakness, improvement followed; apparently normal in 14 minutes. Died overnight	
,,	,,	,,		10 c.c.			Collapse after inoculation. Dead in 2 hours	
							Collapse after inoculation, no improvement, chloroformed at the end of 3 hours	
W. 58	A few mins.							
,,	,,	Small intestine	5 c.c.				None	No evidence of *B. paludis* toxin
,,	,,	,,	10 c.c.				No immediate symptoms but animal died overnight	

within a short time of making the test and so permitted the testing of liquids which were not sterile. The contents of the alimentary canal were, even when diluted with saline, frequently of such a nature that they defied the methods of filtration at one's command. When filtration was impracticable the material was centrifuged and the supernatant fluid without further treatment tested for toxin by inoculating it intravenously into rabbits.

Material from the alimentary tract of five sheep, all of which were typical cases of "struck," has been examined for *B. paludis* toxin. In one case, the toxin was demonstrated in the contents of the abomasum and in another in the contents of the small intestine.

In a third case, Sheep No. 57, a toxin was present in the filtered contents of the small intestine, but its identity was not established.

Unless the toxin was present in a concentration sufficient to kill a rabbit within a few minutes of intravenous inoculation, attempts to establish the specificity of the toxin were not made. It is now thought that too rigorous a standard was set, especially when filtered material was being dealt with, as small doses of *B. paludis* toxin when injected intravenously into rabbits may not kill the animals until 24 to 48 hours have elapsed.

The effects produced upon rabbits by the inoculation of the material from Sheep 57 were in entire agreement with what would have been expected had small quantities of *B. paludis* toxin been inoculated. It is therefore probable that *B. paludis* toxin was present in the contents of the small intestine of Sheep 57.

In the two remaining cases, there was no evidence of *B. paludis* toxin being present.

Search has also been made for *B. paludis* toxin in the contents of the alimentary canal in two experimental animals. One had been infected by feeding 3½ litres of broth culture and the other was infected by feeding the bacteria from 3½ litres of culture suspended in nutrient broth, but in neither of these cases was *B. paludis* toxin demonstrated.

B. paludis *Toxin in the Peritoneal Fluid of "Struck" Sheep.*

The marked injection of and hæmorrhages from the peritoneal vessels, and the turbid effusions of fluid in the peritoneal cavity, the turbidity being due to leucocytes, suggested that these were produced by a localised inflammatory reaction independent of or supplementary to any toxæmia.

Peritoneal fluid was examined for *B. paludis* toxin by inoculating amounts varying from 5 to 12 c.c. intravenously into rabbits.

The toxicity of the peritoneal fluid of Sheep W. 42 has already been noted, the toxin being present in high concentration. But this animal had been dead for six hours, and a very rich culture of the bacteria was found in the fluid. There is little doubt that the toxin was attributable to the bacteria present in the fluid.

Sheep W. 53 presented an interesting case of the absence of toxin from the peritoneal fluid even though bacteria were present in smears

made from the fluid, and no toxin was demonstrated in the peritoneal fluid of two sheep, W. 49 and W. 57, although *B. paludis* was cultivated from the fluid. It would, therefore, appear that small or even moderate numbers of bacteria do not give rise to demonstrable amounts of toxin.

Toxin was demonstrated in 10 c.c. amounts of the peritoneal fluid from Sheep W. 54. The fluid was collected within a few minutes of death and no bacteria could be found in smears. The toxin was neutralised by *B. paludis* antiserum, but not by *B. welchii* antiserum. To find *B. paludis* toxin in the body at the time of death is alone strong corroborative evidence that the toxin is the cause of death, but to find toxin in the peritoneal fluid under conditions where it is not reasonable to attribute its presence to the very scanty number of bacteria in that fluid is remarkable.

Toxin, but in much higher concentration, was found in the abomasal contents of Sheep W. 54.

The presence of toxin in the peritoneal cavity at some stage of the disease would offer an explanation or the peritonitis which is such a constant feature of the disease. The absence of toxin at any one arbitary moment cannot be regarded as an indication that toxin has not been present ; dilution, absorption, and adsorption may have occurred and toxin be no longer demonstrable.

Toxin has been demonstrated in the peritoneal fluid of two experimental sheep, the one fed with bacteria suspended in $3\frac{1}{2}$ litres of nutrient broth and the other fed with $4\frac{1}{2}$ litres of culture. In neither of these sheep were bacteria present in the peritoneal fluid in sufficient numbers to be found in smears. In one case 50 c.c. of citrated peritoneal fluid was centrifuged and the deposit examined, but no bacteria were found. This case illustrates the impossibility of attributing the toxin to bacterial growth in the peritoneal cavity.

If toxin did not originate in the peritoneal cavity it may have been derived from the blood stream or direct from the intestine. Had the toxin been derived from the blood it would be expected in the transudates in both the peritoneal cavity and the thoracic cavity, but this was not the case. When the distinctive and very constant inflammatory reaction of the peritoneum as opposed to the absence of a comparable reaction in the other great serous cavities is considered, it makes it difficult to attribute the peritoneal lesions to anything but a direct passage of toxin from the intestine to the peritoneal cavity—a passage which would be facilitated by the extensive injury inflicted upon the intestine.

Bacteria may be found in the peritoneal fluid while the heart blood appears to be sterile. May not toxin precede the passage of bacteria from the intestine to the peritoneum ?

Toxin may at the same time be absorbed from the intestines by the blood and lymph stream, but the absence of lesions in the liver and the frequent normal appearance of the mesenteric lymph glands would indicate that this does not occur to a marked degree.

General Summary and Conclusions.

The present paper deals with a specific disease of the sheep characterised by an acute and fatal enteritis, peritonitis, and toxæmia. The disease is seasonal, occurring mainly during the late winter and spring months.

The lesions encountered are as follows : Inflammation of the small intestine and occasionally of the abomasum. Ulceration of the small intestine is frequently found, and the cæcum and large colon are sometimes in a state of contraction, their mucous membrane being thrown into folds obliterating the lumen of the bowel. Extensive transudations of fluid are found in the peritoneal cavity, together with injection of and hæmorrhages from the peritoneal vessels. Degenerative changes are occasionally evident in the the kidneys, and transudations of fluid may be present in the thoracic cavity and in the pericardium.

The tissues and body fluids may be sterile, but frequently *B. paludis* may be cultivated from some of them.

As the interval between death and the post-mortem examination increases so, concurrently with post-mortem changes, do the numbers of *B. paludis* increase. Varieties of micro-organisms appear in the peritoneal fluid, but pure and very rich cultures of *B. paludis* are generally present in the muscular tissues even when the post-mortem changes are far advanced. A characteristic and almost specific post-mortem change is met with in the subcutaneous, intermuscular, and muscular tissues. This simulates in a remarkable manner the characteristic ante-mortem changes found in the affected areas in black-quarter of the bovine species. These post-mortem changes in sheep have given rise to the erroneous idea that the disease is a true black-quarter.

The lesions found at the time of death, with the exception of the ulceration in the intestine, have not been attributable to a bacterial invasion of the body tissues. The primary lesion is a necrosis of the superficial mucous membrane in the intestine, but this is not caused by bacteria invading the tissues. The disease is not an inoculable one in the ordinary sense of the term. The disease has been produced by feeding large quantities of broth cultures of *B. paludis* and by feeding the bacteria suspended in fresh nutrient broth.

In natural cases of the disease *B. Paludis* toxin has been demonstrated in the contents of the alimentary tract, and in natural and experimental cases evidence of the passage of this toxin into the body is available, the toxin having been found in the peritoneal fluid under conditions which preclude the production of that toxin in the peritoneal cavity, which suggests that it was derived directly from the contents of the alimentary canal.

The enteritis and peritonitis and toxæmia are ascribable to *B. paludis* toxin derived from the alimentary canal.

Prior to death there is frequently a moderate bacterial invasion

of the body, but this is not the cause of the lesions or the primary cause of the peritonitis or of the toxæmia.

Bacteria may play a secondary part in the ulcerative process in the small intestine.

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AN OUTBREAK OF DISEASE AMONGST IMPORTED PEDIGREE CATTLE AT THE VETERINARY QUARANTINE STATION, NAIROBI.

By R. W. M. Mettam, M.Sc., M.R.C.V.S.

Introduction.

During the summer of 1929 a serious mortality occurred amongst valuable imported pedigree cattle of many breeds and of both sexes which were undergoing immunisation against redwater and anaplasmosis at the veterinary quarantine station, Nairobi.

Previously the station had shown a satisfactory record as far as the health of the bovine inmates was concerned. From time to time an occasional animal died from anaplasmosis as result of vaccination. An occurrence of this nature is not unexpected since a certain percentage of the inoculated animals react violently to the vaccine and the less resistant ones die.

The sudden appearance of the disease described in this paper was not foreseen, and when ten animals were simultaneously affected it was originally feared that an infectious disease had gained entrance into the station. The high mortality and the alarming symptoms soon convinced everyone who saw the cases that a new disease had made its appearance. For a time great alarm existed in the colony, especially amongst stock owners, and the heavy financial loss entailed by the death of so many highly insured animals led up to the initiation of the present investigation.

A detailed history of the quarantine station will not be given in this paper. It is only necessary to mention here that, at the time the disease now being described first appeared, there were seventy head of cattle in the station in the process of being immunised against redwater and anaplasmosis. All the animals had just arrived at the quarantine station from Great Britain. The sea voyage had lasted for a month, and on arrival at Mombasa they were immediately railed to Nairobi. The time from the date of leaving England to the arrival at the quarantine station therefore did not exceed 35 days.

There was no history that any serious mortality had ever occurred amongst previous batches of animals in the station. In 1928, however, two deaths occurred which could not be attributed to anaplasmosis. Judging from the accounts of the post mortems held on these animals there is no doubt that both of them died from the complaint which was to cause such a heavy mortality in the following year.

History of the Animals Affected.

In Table I the history of some of the cases is set out. They are selected because they are typical of the disease and because it is clearly impossible to describe the history of every affected animal in detail.

It will at once be noticed that Ayrshire heifer No. 27 had not been immunised like the other cattle. This animal was found to be heavy in calf and, in order to avoid any risk of abortion, her vaccination was postponed. Nevertheless she contracted the disease and died exactly a month after calving. It is extremely difficult to explain the origin of the disease in this animal. Every possible care was taken to nurse her over her parturition, which she went through normally. She had been isolated from the other cattle on account of her physical condition the whole time she was in the station. It seems impossible to escape the conviction she was not contaminated during her puerperium.

Be that as it may, other conclusions which may be drawn from this case are (*a*) that the disease can appear when there is no history of close contact between sick and healthy animals, and (*b*) that the pathogenic agent, whatever its nature, was not conveyed by means of the blood vaccine.

Originally it was believed that some virus might have been accidentally inoculated into the quarantine animals, but further investigation finally proved that there was no direct or immediate association between the vaccine and the pathogenesis of the disease. The case of heifer No. 27 finally dispelled this hypothesis.

The following deductions can also be made from Table 1 :—

1. That with one exception (Heifer No. 27 noticed above) the disease appeared in animals which had received either the first or both inoculations of the redwater anaplasmosis vaccine. Symptoms might appear as early as the ninth or tenth day after vaccination or they might be very much delayed, *e.g.*, in one case until the 98th day.

2. That in one animal which recovered from the disease the second vaccine was tolerated.

3. That the disease appeared in animals which reacted variously to the vaccines.

The Diet of the Quarantine Animals.

Each animal received a daily ration of 18-20 lbs. of sun dried lucerne and 4 lbs. each of crushed maize and bran. A salt lick was also provided. No fresh green food of any kind was fed to the animals.

It was believed by certain dietetists that this feed, though adequate in amount, was dangerously low in vitamins and other fresh food principles. This opinion led to the examination of the possibility that the disease was a form of avitaminosis. Similarly, the preponderance of dried lucerne in the feed caused an investigation to be made into its origin and suitability as an article of diet for sick animals, and, further, to ascertain whether it might not be solely responsible for the disease, which has a marked resemblance to the condition described in the literature as sweet clover disease. These subjects are again discussed later in the paper under the heading of differential diagnosis.

TABLE I.

TABLE SHOWING HISTORY OF THE DISEASE AND ITS RELATION TO THE REDWATER-ANAPLASMOSIS VACCINE.

No. and Sex.	*History in Quarantine.*	*Reaction to Vaccine.*	*History of Quarantine Disease. Remarks.*
Ayrshire Bull No. 32	Admitted 20/11/29. 1st vaccine 23/11/28. Re-inoculated with 1st vaccine on 11/1/29. 2nd vaccine 5/2/29	Reaction to 1st vaccine was so poor that a second dose was given, after which there was a good reaction to *A. centrale*. At the time the 2nd vaccine was inoculated the animal appeared normal	The disease ran a course of six days, death occurring nine days after inoculation of the 2nd vaccine. This case was typical of the disease at post-mortem.
Blue Albion Bull No. 11	Admitted 20/11/28. 1st vaccine 20/11/28. 2nd vaccine 25/1/29	Reaction was so poor that the animal appeared to possess considerable immunity. At time of inoculation of the 2nd vaccine the animal was quite healthy	Death occurred nine days after inoculation of 2nd vaccine. First symptoms of illness appeared the day following second vaccination. The post-mortem was typical of the disease.
Ayrshire Heifer No. 27	Admitted 20/11/28. Not vaccinated as " in calf." Calved 5/1/29	———	12 days after calving showed a sharp febrile reaction, the temperature fluctuating around 105° F. Death occurred on 4/2/29 after an illness of eleven days. The post-mortem was typical of the disease
Shorthorn Bull No. 20	Admitted 20/11/28. 1st vaccine 27/11/28. Re-vaccinated with 1st vaccine 11/1/29	There was no reaction to 1st vaccine. After re-inoculation a mild fever was observed only on two days while *A. centrale* was very rare. No redwater reaction	Ten days after reaction noted in previous column a severe epistaxis occurred with the appearance of generalised purpuric spots. Course of disease was 15 days, and the post-mortem was very characteristic.

Ayrshire Bull No. 31	Admitted 20/11/28. 1st vaccine 27/11/28	Redwater parasites were always rare and reaction was mild. *Anaplasma* first seen on 40th day	Purpuric hæmorrhages were first observed 35 days after vaccination. The fatal disease ran a peracute course of six days. The post-mortem was quite typical.
Red Poll Bull No. 41	Admitted 20/11/28. 1st vaccine 13/12/28. 2nd vaccine 23/5/29	Reaction to 1st vaccine was very acute and prolonged ; *A. centrale* was present in large numbers for 42 days. Subsequently there was a high temperature for three weeks for no obvious reason	Purpuric hæmorrhages appeared 98 days after vaccination. They disappeared under adrenalin treatment in 12 days. Five weeks later the 2nd vaccine was given, to which there was a normal reaction and the animal was subsequently sent to its owner where it has enjoyed very good health
Shorthorn Bull No. 15	Admitted 20/11/28. 1st vaccine 20/11/28. 2nd vaccine 25/1/29	There was a normal redwater reaction but a severe one to *A. centrale*. Reaction to 2nd vaccine was mild	The animal showed generalised purpuric hæmorrhages which persisted for 31 days. These appeared 25 days after the 2nd vaccine. Recovered under adrenalin treatment. No relapse reported.
Red Poll Bull No. (?)	Admitted 20/11/28. 1st vaccine 13/12/28. 2nd vaccine 5/2/29	Severe reaction to first vaccine, but the animal was in good health at the time of the 2nd inoculation	Purpuric hæmorrhages appeared nine days after inoculation of the 2nd vaccine. The disease ran a very rapid course, lasting only five days. The post-mortem was quite typical

Symptology, Course, and Mortality.

The onset of the disease was very insidious. The first indication that something was wrong was the appearance of very noticeable red hæmorrhages in the skin and on the visible mucosæ. These cutaneous hæmorrhages were best seen on the sparsely haired or naked areas of the body, *e.g.*, scrotum or mammary glands, perineum, anus, muzzle, ears, and medial aspect of the thighs. Careful examination, however, showed that similar hæmorrhages were generally present all over the body. On the mucosæ they were in the form of petechiæ, diffusely scattered and bright red in colour. They were particularly numerous on the nasal mucous membrane, but they also existed on the mucosa of the mouth, throat, vagina, vulva, rectum, and conjunctiva.

There was a slight discharge from the eyes and nostrils which at first was of a serous nature. Within a few days it became ropy and blood-tinged, especially that coming from the nose. The animal constantly blew small quantities of blood-stained secretion out of its nasal passages.

At first there was obstinate constipation but after a few days blood was noticed in the fæces. In some cases this was the first symptom of illness and preceded the appearance of skin lesions. The blood was either bright red in colour or very dark, giving the fæces a mashy, tarry appearance. Once blood came away from the intestines the fæces became soft and pultaceous and had a somewhat offensive odour. The blood was clotted and in masses larger than a clenched fist, and either mixed with mucus or shreds of mucosa or in the form of red streaks. As the intestinal hæmorrhages increased in severity the fæces became very fluid and were ejected with a great force from the rectum on to the walls and stable fittings.

Often the dysentery was not profuse. In these cases there was generally very pronounced tenemus followed by the evacuation of small clots of dark bloody mucus or pure blood. This act was accompanied by much groaning and grinding of teeth, while the animal stood back with head depressed and back arched.

At this stage a heavy, sickly, disagreeable odour emanated from the body of the animal, while the fæces and the breath became very foul. In a few cases long shreds of intestinal mucosa forming a cast of the bowel were passed per rectum.

A very remarkable clinical feature was the delayed coagulation time of the blood. Unfortunately no biochemical observations were made, but it was very noticeable that even small skin wounds, such as were made on the ear to obtain a drop of blood for the preparation of slides for routine blood examination, bled freely and showed so little tendency to clot that the animal stood in a small pool of blood formed as the result of constant drippings from the ear. Similarly after jugular venesection bleeding

continued to take place both into the perivenous tissues and on to the exterior.

It is impossible to state definitely whether this condition was complicated by fever, as, in most cases, the patient had not yet completely recovered from its anaplasma reaction or was still in a pyrexial condition due to the reaction. In this connection the history of Ayrshire heifer No. 27 is of some interest. As already stated, this animal was not vaccinated after she arrived at the quarantine station as she was heavy in calf. She calved eight weeks after her arrival at the station and during that time ran a steady temperature. Three weeks after calving her morning temperature began to rise, reaching 105° F. on the fifth day, after which it fluctuated between 104° and 105° F. for a further six days, when death occurred. On post-mortem this animal showed typical lesions of the disease. It may be added that certain other animals, which had clinically finished their vaccination reaction, showed a sharp rise in temperature when the hæmorrhagic syndrome was at its worst a few days before death. It seemed reasonable, therefore, to infer from these observations that the disease was accompanied by a mild febrile reaction.

Course.

In every untreated case the course of the disease progressed towards a fatal issue, but after the introduction of adrenalin as a curative the mortality was arrested. The course of the disease in treated and untreated cases was therefore different.

(*a*) In untreated cases the course of the disease varied in duration but always ended in death. The symptoms became worse and the animal rapidly weakened as the result of continual hæmorrhages. Anorexia was complete and recourse had to be made to the use of nutritious drenches to help to maintain the strength of the patient.

The intestinal symptoms were so pronounced that the animal appeared to bleed to death through its intestinal mucosa. Death was preceded by great prostration and weakness though the animal never showed signs of coma. Respirations were very hurried, of a blowing nature and accompanied by groaning and moaning. The pulse, at the rate of 80-100 per minute, was very weak and at times imperceptible, while there was continual grinding of the teeth. At the time of death the animal was so feeble that it could hardly struggle. The pallid visible mucosæ were typical of severe internal hæmorrhage and showed numerous petechiæ. In most cases during the last few days of life œdema of the limbs, lower aspect of of the trunk, and neck was so great that animal refused to rise. There was also a transudation of straw-coloured fluid through the apparently intact skin. This tended to dry and form scales or crusts over the œdematous areas.

The duration of the disease from the first appearance of the

hæmorrhages to death varied between 4 and 11 days, and the mortality before the use of adrenalin was 100 per cent.

(*b*) In treated cases. Whatever may have been the real therapeutic action of adrenalin rapid improvement and recovery followed its employment. Within 14 days the visible mucosæ became cleared of all hæmorrhages and the epistaxis subsided in 24-48 hours. The dysentery was controlled, and after a week blood was no longer observed in the fæces, which thereupon recovered their soft consistence and normal odour.

The skin lesions were refractory and slow in clearing up. The hæmorrhages, at first red in colour, turned purplish, and finally the entire skin assumed a pale yellow colour. There was much epithelial desquamation and the hairs fell out in abundance, leaving extensive, bald, dry, corrugated areas covered with greasy, scaly scurf. Even with the aid of skin tonics and hair restoratives (vide treatment), it took three months or more for the hair to grow and cover the bald patches.

The Pathology of the Disease.

(*a*) *Morbid Anatomy.*

The carcases were generally in fair condition and gave off a heavy, disagreeable, sudorific or uræmic odour. Rigor mortis appeared very rapidly and was very pronounced. Except for the presence of hæmorrhages, the visible mucosæ were very pale and bloodless. In many cases there was a bloody, slimy, nasal discharge, while the anal orifice was dilated or plugged by a mass of dark blood-stained mucus. In one animal fresh red blood trickled from the anus.

Except in one case, cutaneous hæmorrhages were found on every part of the body, especially noticeable on the hairless parts, *e.g.*, perineum and sheath. Here and there were alopectic areas where the hair had been shed and where the skin was nodular, corrugated, raw, and covered by a bloody or serous exudation. In more advanced cases the skin was cracked, thickened, and scurfy. The lesions varied in size up to one square foot, but generally they were as large as the palm of the hand.

Very remarkable subcutaneous changes were present in every case. The subcutaneous, areolar, and intermuscular tissues were profusely infiltrated with œdematous, hæmorrhagic œdematous, or purely hæmorrhagic extravasations, which varied considerably over 12 inches in length and six inches in thickness. When these extravasations were purely hæmorrhagic they resembled a large hæmatoma or the lesions of some anærobic infection, from which, however, they could be readily differentiated bacteriologically. When free from an admixture of blood they were gelatinous, soft, and straw-coloured. Such lesions were noticed over the flanks, along the abdomen, in the axillæ, over the shoulders and neck, and along the medial aspect of the thigh, and were the cause of

the œdematous swellings of the dependent parts noticed during life. Although they were most pronounced in the subcutaneous tissues similar lesions could be traced into the connective tissue septa between the muscles, while the musculature itself was very dark in colour but free from any gassy or necrotic lesions.

Sections through the red areas on the skin showed that they resulted from hæmorrhages into the hair follicles, which consequently became swollen and nodular. Subsequently the hairs fell out and the superficial layers of epithelium were shed, forming a greasy scaly dandruff.

The buccal mucosa was very pale but shewed diffuse submucous hæmorrhages which varied in size. The large ones, measuring 3 cm. along their long axis, were found on the tongue and lips. The gums showed scattered ecchymoses but the teeth appeared normal. Similar hæmorrhagic spots were observed on the mucosa of the pharynx and œsophagus. There was always pharyngitis, the mucosa being reddish purple, swollen, and covered with catarrhal material.

The Stomachs.

The first, second, and third stomachs were normal, though in the heifer referred to previously the submucosa of the omasal-abomasal orifice was swollen and severely infiltrated with a red, gelatinous, œdematous fluid.

The abomasum was gravely affected. The mucosa was either a deep rose pink colour or a dirty slaty grey, but it was always greatly swollen, soft and jelly-like, particularly the laminæ which were greatly enlarged. The submucous tissues were infiltrated with a soft gelatinous fluid the colour of red currant jelly. This infiltration was as a rule spread throughout the organ, in one animal even into the omasum. In a few cases it was localised but the laminæ were always affected. In two animals the infiltration was so profuse that the wall of the abomasum was 2-3 times thicker than normal. On one occasion the mucosa showed the presence of small ulcers which on an average measured ·75 cm. in diameter. They had an angry appearance, with a black jagged border, and were very superficial in their distribution along the mucosa. The abomasal contents were always fluid and contained much blood.

The Small Intestines.

Ulceration was never noticed, but numerous punctiform hæmorrhages were present through the gut. Capillary stasis in the form of rich red arborescences was more often noticed than genuine red hæmorrhages. The mucosa was covered by a creamy pink catarrhal material. In the submucosa there was a hæmorrhagic infiltration similar to that noticed in the abomasum. The bowel contents were fluid and mixed with bright blood. The lesions were least pronounced in the ileum.

The Large Intestines.

This part of the bowel was less gravely affected than the small intestines. Submucous hæmorrhagic infiltration was only noticed in the cæcum and coils of the colon. No ulceration was noticed, and the colour of the mucosa was a deep rose, indicating a moderate degree of stasis. The rugæ of the rectum were closely bespattered with punctiform hæmorrhages. The bowel contents varied; often they were firm and contained much dark blood, or the blood was bright red. Shreds of mucosa were often found in the lumen of the rectum, while long casts of the intestinal mucosa was recovered from the rectum of Bull No. 20.

Mesenteric Glands.

These were always swollen, juicy, pale in colour, and exuded a pinkish creamy fluid.

The Peritoneal cavity was either quite empty or contained thick blood-stained fluid, which measured up to a litre. Hæmorrhages were also present. They were either rare and confined to small areas of parietal or visceral peritoneum or present as scattered petechiæ, which were very numerous, large, and varied in size from a pin head up to a diffuse extravasation. In every case examined the serous surface of the abomasum, omasum, adjacent surface of the diaphragm, and rumen were occupied by a diffuse, soft, dark red suffusion, and a similar lesion was present on the opposing parietal serosa. In one bull there was an extravasation as large as a man's head—a veritable hæmatoma—which was undergoing fibrous organisation as indicated by the appearance of soft adhesions between the abomasum, diaphragm, omasum, and rumen and the overlying parietes. In the worst cases the serosa of the intestines, diaphragm, mesentery, and omentum were literally besprinkled with blood.

Spleen.—There were bright red hæmorrhages varying in size from small petechiæ to extravasations as large as the thumb nail under the capsule. On section the pulp was dryish, somewhat swollen, and reddish brown in colour. Generally the malpighian corpuscles were pale, large, and prominent.

In the heifer there was marked *tumor splenis,* but the corpuscles were not unduly prominent though the pulp was swollen and rich in blood. Very frequently there were soft, pinkish, fibrinous deposits on the surface of the capsule.

The Liver showed no constant lesions. In a few cases subcapsular hæmorrhages were noticed but they were very seldom larger than a pea.

Generally the organ was very friable, light brown in colour and contained little blood. In one bull the parenchyma showed bright red areas in many of the lobules, some of which were completely filled. Microscopically these were due to extreme stasis of

blood in the liver lobules. In many cases there were isolated "nutmeg" areas.

The bile was viscid, flaky, and the colour of golden syrup. The mucosa of the gall bladder was swollen and varied in colour from a rose pink to a deep red as result of marked stasis of the vessels in the submucosa. No ulceration was observed.

The Urinary Apparatus.

(*a*) The capsule stripped easily off the kidneys. On section the cortex was swollen, friable, and generally pale in colour. Here and there were isolated dot-like cortical hæmorrhages. In many places the cortex was distinctly "nutmeg."

(*b*) There were generally small subserous and submucous hæmorrhages in the bladder, or else the mucosa presented a rosy flush. The urine when present was normal, but unfortunately it was not collected for chemical examination.

The Sexual Organs.

(*a*) In the heifer the uterus, vagina, and udder appeared normal. There were small subserous hæmorrhages in the uterine broad ligament.

(*b*) The genital organs in the male never presented any lesions.

The Respiratory System.

(*a*) There were numerous scattered miliary hæmorrhages on the nasal mucosa. Blood and mucus were present in the passages. In two animals the larynx and trachea were normal, but otherwise these organs showed numerous submucous petechiæ and a slimy straw-coloured fluid in the passages. In one bull there were considerable hæmorrhagic infiltrations of the peritracheal tissues.

(*b*) The pleura and contents of the pleural cavity were normal in every case except in the heifer, in which there was a soft rounded blood clot as large as a man's head, lying on the sternum in the middle mediastinum between the main lobes of the two lungs. The clot was dark in colour and when moved was found to be partly composed of dark fluid blood. In places it appeared to be undergoing early organisation, for it showed a fibrinous covering and fibrinous trabeculæ. It was largely covered by fat derived from the suprasternal adipose deposit. The aorta, venæ cavæ, and pulmonary vessels were always quite normal.

(*c*) Subpleural and interstitial emphysema were common lesions. The lungs were generally considerably inflated and appeared too large for the chest cavity, showing well marked costal impressions.

Except for the hypostasis and commencing red hepatisation that could always be traced to prolonged decubitus, the lungs showed no specific lesions beyond slight œdema and unusual bloodlessness.

Blood Vascular System.

(*a*) The blood was fluid and had lost its clotting powers. In the veins and cardiac ventricles it formed very soft soggy coagula. It appeared to be hæmolysed and stained very deeply. No naked eye lesions could be found in any of the blood vessels.

(*b*) In two bulls and in the heifer there was 100-200 c.c. blood in the pericardial cavity. In one animal the entire cavity was occupied by a soft, dark-red coagulum to which the pericardium was intimately connected by numerous delicate pinkish filaments which apparently had been formed by organisation of the clot.

(*c*) Cardiac hæmorrhages were quite typical. They varied in size from a pin head to a diffuse extravasation and in places were 1 cm. thick. They existed under both the endocardium and epicardium, giving the organ a reddish-black blotchy appearance. The cardiac fat was soft, gelatinous, and pinkish in colour. In more mild cases the hæmorrhages were chiefly found along the *sulci coronarii,* and in every case there were scattered small pinkish deposits of fibrin on the epicardium. Petechiæ were noticed on the atrio-ventricular and semilunar valves.

The myocardium was swollen and moist but firm. In colour it varied from a light mahogany-brown to a watery pink. It showed linear or streaky red areas the size of a split pea or up to 1 cm. in length. In the heifer the heart muscle was somewhat friable and had a hyaline appearance.

Brain and Nervous System.—Appeared normal.

(*b*) *Pathological Changes in the Blood During Life.*

Unfortunately no physico-chemical investigations were carried out on the blood during life, and no explanation can be offered of the delayed coagulation time of the blood, which was such a characteristic clinical feature of the disease. Further, as every animal except the heifer was either going through its redwater-anaplasmosis reaction or had not completely recovered from it, the blood picture was possibly obscured by the pathological changes caused by the protozoa. In the heifer, however, in stained films the red and white blood elements showed no abnormal characters at the time the animal was suffering from the hæmorrhages which ultimately caused its death. It is, of course, dangerous to dogmatise from one observation, but it may be suggested that there were no gross pathological changes in the blood during the disease.

(*c*) *Pathological Histology.*

In the solid viscera the capillaries and smaller arterioles showed very marked stasis of blood, the vessels being filled much beyond their normal capacity. As a result, there was considerable distortion and disorganisation of the organic elements. Cloudy swelling and fatty degeneration were found in the liver, kidneys, and

myocardium, but it is possible that these were due, in part at any rate, to the concomitant protozoan infection caused by the vaccine.

In no instance were any fatty changes observed in the endothelial cells of any of the blood vessels of either the viscera or the serous and mucous membranes. There was no discernible microscopic lesion which would permit of the leakage of blood into the perivascular tissues.

In the case of the serosæ and mucosæ more or less considerable quantities of blood had escaped out of the vessels. Such hæmorrhages varied in size from a small collection of erythrocytes up to a vast suggilation of which only a small area could be prepared for histological examination. The microscopic appearance of these lesions was similar to that of a blood clot, though the amount of fibrin present as demonstrated by Weigert's stain appeared to be very small. In material taken from subacute cases there was an obvious increase in the amount of peripheral fibrous tissue, as if an attempt was being made to encapsulate the hæmorrhage.

A noticeable feature about the histological appearance of the various organs and tissues in acute cases was the marked absence of any inflammatory phenomena.

No data are available to indicate the fate of those large hæmorrhages in the animals that recovered under treatment. The very marked icteric discolouration of the skin of convalescents would apparently indicate that a considerable amount of pigment, probably of the nature of hæmosiderin, was liberated but, unfortunately, no observations were made on these animals.

Etiology.

The cause of the disease remains obscure. Blood cultures made during life or from the heart blood at death were invariably sterile. Streptococci and Gram-negative lactose fermenters were recovered from the alimentary submucosa, liver, spleen, and kidneys of bull No. 42, bull No. 20, and heifer No. 27.

The streptococcus was Gram-negative; it fermented dextrose, saccharose, lactose, inulin, raffinose, and salicin. On blood agar slight hæmolytic zones appeared about the colonies, *i.e.*, they were weak Beta types. In every way the organism resembled the typical bovine fæcal streptococcus (type B) described by Ayres[10] and Mudge. It was non-pathogenic for cattle and rabbits.

The Gram-negative lactose fermenter bore the characters of *B. coli communis*. The organism was slightly pathogenic for cattle, as intravenous inoculations of an emulsion of a culture killed two animals in 18 and 21 days respectively, without, however, the development of any purpuric symptoms.

Other bacteria isolated included *Staphylococcus albus, aureus,* and *citreus*; *B. proteus;* and *B. pyocyaneus,* none of which could be inculpated as the causal pathogen.

Inoculation of blood from well marked clinical cases of the disease proved negative for equines, sheep, rabbits, and other

cattle (see Appendix). Exhaustive research also failed to reveal the presence of any protozoa or filterable virus as the possible etiological agent. The disease was not conveyed by actual contact and arthropod vectors could be excluded.

Anaphylaxis was soon ruled out of consideration as the disease was observed in one non-vaccinated animal and in an animal which had only received its first inoculation.

Differential Diagnosis and Discussion.

The two diseases described in the literature to which the quarantine disease can be compared in detail are (*a*) sweet clover poisoning, and (*b*) that heterogeneous group known as hæmorrhagic diatheses, which include scurvy and purpura hæmorrhagica. It will be necessary to discuss the relation of these obscure conditions to the quarantine mortality, since the exclusion of a biological etiological factor seemed to point to either the possibility of some noxa being introduced into the body with the food, as in sweet clover poisoning, or some little understood lesion of the hæmopoietic tissues, such as might result from avitaminosis or interference with the normal regulating activities of the spleen or recticulo-endothelial system.

At one time the possibility of a mineral or animal poison was considered. The former was quickly dispelled by analytical examination of morbid material, while the extent and severity of the pathological lesions observed in the quarantine disease were clearly unlike any that had ever been described in poisoning by venomous snakes. Poisonous serpents were never actually found in the cattle byres, though the puff adder (*Bitis gabonica*) is frequently met with on the Athi plains in the vicinity of the quarantine station.

Similarly, it was thought that some cardio-vascular poison of vegetable origin might have been used maliciously by natives who had a grudge against European authority. Many settlers in Kenya believe that their stock is often wilfully poisoned by native employees who bear a grievance against them. Unfortunately, it is at present impossible to diagnose cases of vegetable poisoning by mere analysis of pathological material, as no reliable chemical tests exist for detection of some of the commonly used but little known vegetable poisons of tropical Africa.

The investigation into the quarantine disease happened to coincide with certain toxicological tests that were being conducted at the Kabete Laboratory on some of the better known African arrow poisons and the opportunity was taken to compare the pathology of the two conditions. It was soon apparent that the microscopic anatomy of the quarantine disease was not produced by any irritating poison in which category most of the native vegetable poisons of Kenya can be placed.

Sweet Clover Disease.

This disease has been described in North America by Schofield[4], Roderick[5] and Schalk[6] and is said to cause serious loss every year west of the Alleghany mountains. Schalk has noticed its occurrence in animals fed " on specimens of hay and silage that were moulded or at least possessed some degree of spoilage or damage "; and it is further noted that " not all moulded, spoilt or damaged clover, hay, and silage possess the disease producing qualities." He did not observe the disease to follow eating good, clean, well preserved sweet clover, hay, or silage. Schofield was inclined to blame " certain moulds " as the causal agent, but Schalk was more careful and qualified the above quotations in his paper by adding that " unfortunately the disease factor in the forage cannot be detected by either layman or scientist." It appears from the work of these authors that sweet clover (*Melilotus alba*) and even ordinary hay, under certain circumstances not clearly understood but apparently as result of improper drying or treatment in the silo, becomes toxic for cattle and capable of causing well marked clinical symptoms and pathological lesions.

Although sweet clover was not fed to the quarantine animals its near relative lucerne was the staple article in the diet. Recently reports have appeared in the literature on the toxicity of lucerne (*Medicago sativa*) under certain conditions. Thus, an eczematous inflammation of the pigmented portions of the skin has followed exclusive feeding on this plant, especially in India and Australia, where the animals are exposed to hot sunshine. The skin irritation was most intense during the day and disappeared at night but only to reappear on the following morning. This sounds very much like " photo-sensitisation " which has been definitely shown to follow ingestion of buckwheat (*Fagopyrum esculentum*) under irridiation or intense sunlight. In this condition there was very marked irritation of the skin, urticaria, petechial hæmorrhages throughout the body, and parenchymatous degeneration in various viscera. Shead[11], Caylor and Schlotthauer, in severe experimental cases, noticed " marked increases in the coagulability of the blood " without development of any other pathological lesions.

All these observations proved to be of great interest and led to a careful examination of the lucerne that was being used as the chief cattle food at the quarantine. It was at no time believed that photo-sensitisation had anything to do with the quarantine disease, since the experience of Shead and his co-workers on the coagulation of the blood in their experiments was so fundamentally different from what was noticed in the Nairobi cattle.

The possibility of a mould or some such agent in the lucerne was carefully looked for, and the condition of the baled product, which was bought in large amounts by the Government, was thoroughly examined. On no occasion was any questionable material discovered, and it was subsequently found that large

consignments of the same cutting of the lucerne were used by other stock farmers in the colony without any apparent ill-results.

The description of the pathology of sweet clover disease by Roderick[5] was so similar to what was observed in the Nairobi cattle that it was thought necessary to exclude the possibility of poisoning by damaged fodder.

In the following table sweet clover poisoning and the quarantine disease are compared very briefly as regards their symptomology and pathology. It is impossible to avoid commenting on the very close similarities presented by these two diseases.

The "Hæmorrhagic Diatheses."

Finally, there are two diseases, at the present time classed by hæmatologists under the heading of hæmorrhagic diatheses, which must now receive attention, namely, scurvy and bovine purpura hæmorrhagica. The former will only receive brief notice, as it is agreed to be a very rare disease of the domestic animals which probably never occurs in bovines under ordinary conditions. The extreme poverty of vitamins in the diet that the animals in the quarantine were receiving suggested that that the disease might be a form of avitaminosis. As will be seen later, this theory could not be substantiated and was abandoned early in the investigation.

(1) *Scurvy* is extremely rare among the domesticated animals and is said to be found only in pigs and dogs. As is now universally known, it is due to lack of vitamin C in the food and can be readily prevented by feeding substances rich in the so-called anti-scorbutic principle, *e.g.*, fresh vegetables, greens, and fruits. It is remarkable that herbivorous animals like cattle should be so refractory in showing symptoms of scurvy when fed for a year or more on a diet in which the anti-scorbutic factor has been destroyed. Theiler,[8] Green and Viljoen, in their research into avitaminosis as a possible cause of lamzietkte, made the following observations, which are of interest, namely, that (*a*) "the vitamin content of green lucerne is high and relatively small quantities of it suffice to protect pigeons against polyneuritis"; (*b*) it seems fairly certain that the exogenous vitamin requirements of cattle are extremely small"; and (*c*) "cattle fed up to periods of 13 lunar months on synthetic rations of exceedingly low vitamin content, as judged by accepted criteria and pigeon analysis, failed to develop either lamziekte or any other disease which could be diagnosed as an avitaminosis." The view is incidently expressed that an avitaminosis in cattle is not likely to occur in practice unless manifested simply in an atrophic form clinically indistinguishable from inanition.

Attempts were made at Kabete to produce scurvy in bovines by feeding over a period of 15 months liberal quantities of lucerne that had been autoclaved at 110° C. for half an hour. No supplementary food of any kind was offered, but, except for symptoms

TABLE II.

	Sweet Clover Disease.	*Quarantine Disease.*
Coagulation time of the blood	Very delayed, may be so slow that the clot phenomenon is absent	Very slow
Presence and nature of subcutaneous swelling	Gait stiff, causing animal to limp while walking. One or more tumour-like subcutaneous swellings over the fleshy parts of the body	Very rarely noticed clinically. Œdema of limbs and belly was noticed in some cases but was never very prominent
Appetite	Normal	Complete anorexia
Period between feeding and first appearance of loss of coagulation power of blood	10-11 days but periods up to 28-42 days occur	Symptoms of illness appeared in animals fed on heavy lucerne ration for at least 70 days. The connection between lucerne and the quarantine ration has not been established.
Site of hæmorrhages	Greatest predilection for subserosæ of pleural and peritoneal cavities and for subcutaneous tissues	Hæmorrhages appear in the submucous, subserous, and interstitial connective tissues
Nature of the disease	Hæmorrhagic, never septicæmic	Hæmorrhagic, never septicæmic
Therapeutic treatment	Drug treatment quite useless. Serum or freh normal bovine defibrinated blood gives very good results	Adrenalin acted like a specific

which resembled those of inanition, *e.g.*, loss of condition, dry harsh erect coat, constant bellowing for food, the animals certainly failed to show the typical scorbutic picture described in text books of human medicine.

All the above observations go to demonstrate the extreme difficulty of setting up experimental scurvy in bovines. Furthermore, although gingival hæmorrhages were observed in the quarantine animals they could not be compared with the pathogenic lesions of scurvy, *viz.*, a swollen, spongy, fungous condition of the gums. It was quite obvious that the quarantine disease was not an avitaminosis.

(2) *Bovine purpura hæmorrhagica.*—This is an extremely obscure disease in veterinary pathology. According to Hutyra and Marek[1], it generally appears without apparent cause and may be observed as a stable infection though transmission from animal to animal has not been established. Walker and Edmonds[9] state that cattle and buffalo (presumably in India) are occasionally attacked by a form of purpura following mastitis or metritis.

The chief contribution to this disease has been made by Rabagliati,[3] who has observed it in Egypt in both Cyprus and Egyptian cattle undergoing hyperimmunisation for the production of anti-rinderpest serum. As a rule purpura appeared 5-9 days after the fourth immunisation, but the period might be delayed as long as the 57th day. Rabagliati, in his second paper, reviewed the literature on bovine purpura and inclines to the theory of platetlet deficiency as the cause of the disease.

It may be remarked in passing that bovine purpura while comparatively common in Egypt is very rare at Kabete, where only exceptional cases of the disease have been observed in animals undergoing hyperimmunisation for anti-rinderpest serum.

In the subjoined table bovine purpura hæmorrhagica as described by Rabagliati in Egypt is compared with the Nairobi quarantine disease both as regards their pathology and symptomology. In many respects the two diseases agree very closely, the most noticeable difference being the marked submucous infiltrations along the alimentary canal, the presence of blood in the serous cavities, and the absence of hæmaturia in the Nairobi disease. It seems as if the two diseases might be fundamentally identical, the severer and more extensive nature of the lesions of the Nairobi disease being explained by the highly bred cattle that were affected in contrast with the coarser Cyprus and Egyptian animals that succumbed to the disease in Egypt. Rabagliati is in a position to explain the pathogenesis of his cases on the theory of blood platelet deficiency since all his animals had recently undergone hyperimmunisation. In the quarantine disease such an explanation cannot be accepted as it does not explain the appearance of the disease in all cases.

Furthermore, if the quarantine disease is really a sequel of

redwater-anaplasmosis vaccination in pedigree stock, it is of great interest to note that no similar condition has ever been described from such countries as Great Britain, South Africa, France, where for many years cattle have been immunised prior to export.

Many theories can easily be formulated to fit all the circumstances of the outbreak, but the writer is of the opinion that the disease was essentially of the nature of a stable infection due to some factor or combination of factors to which both vaccinated and unvaccinated animals were exposed.

Finally, the writer suggests that, until more is known about its pathology, the disease should be considered as an exceedingly virulent type of bovine purpura hæmorrhagica.

Treatment.

Adrenalin hydrochloride (Parke Davis 1-1,000 solution) was the only drug that proved of any benefit in treatment. Given in 5i doses three times a day, either subcutaneously or intravenously, it was attended with rapidly beneficial results. In cases that were obviously bleeding to death larger and more frequent doses were given, and most of such cases responded to treatment in a remarkable manner. Adrenalin is rapidly eliminated from the blood stream, and its action is so transient that repeated doses have to be given to obtain control of severe hæmorrhages. When used on these lines it was instrumental in saving practically every case, and the mortality, which at the beginning of the investigation was 100 per cent., was subsequently greatly reduced.

Physiologically, adrenalin acts as vaso-constrictor and raises the blood pressure by " peripheral stimulation of the vaso-constrictor mechanism of the systemic vessels, and of the accelerator mechanism of the heart " (Sollmann)[10]. Its action is most intense on the arterioles, especially of the voluntary muscles and mucous membranes. It is of interest to note that the inoculation of adrenal extract into normal subjects produces a thrombocytosis, and similar but more temporary results have been recorded following the use of ordinary adrenalin. It is mere speculation to suggest that the beneficial action of the drug in the disease described in this paper may have been due to a thrombocytosis which brought about a restoration of the normal clotting power of the blood.

All cases progressed rapidly under treatment, and during convalescence tonic preparations containing compounds of arsenic, iron, and nux vomica were given several times daily in the food.

A change of diet was given in the form of nutritious drinks and fresh green fodder, such as maize tops and vegetables which were obtained from the native market though, unfortunately, the supply of the latter was very limited.

The skin lesions proved to be very refractory. The best procedure was found to be to wash the skin thoroughly with a warm

TABLE III.

A Comparison of the Pathology and Symptomology of the Quarantine Disease and Bovine Purpura Hæmorrhagica as described by Rabagliati.

	Bovine Purpura Hæmorrhagica.	*Quarantine Disease.*
Nature of the hæmorrhages	Present on visible mucosæ, eyelids, and pharynx. Skin often becomes yellowish. Epistaxis frequent. Delayed clotting of blood from small wounds.	Identical. Yellow discolouration of the skin only observed in convalescents. Epistaxis very severe. Delayed clotting of the blood very noticeable.
Nature of the fæces	Soft and black as if mixed with tar. Large clots of blood may be expelled per anum ; at times even pure blood was discharged	Identical.
Subcutaneous œdematous swellings	Rare. Found over sites of inoculation or along sides of body. Hard and shew no tendency to suppurate or slough. Very slowly absorbed after recovery	Rare. May affect body and limbs, causing stiffness. They are firm and a bloody serous fluid exudes from them. Do not slough.
Hæmaturia	Seen in 3 or 4 cases only. Urine becomes thick and dark as in redwater	Always absent.
Blood smears	Advanced anæmia, with polychromasia, punctate basophilia, anisocytosis, and poikilocytosis	Blood changes always obscured by presence of anaplasma and attendant anæmia,* except in No. 27 in which blood appeared normal.
Serous hæmorrhages	Diffusely scattered, especially on the peritoneum, where, over the rumen, they were as large as soup plates	Identical. Most marked in peritoneum and pericardium. Rare on pleura. The serous cavities contain blood.
Subcutaneous hæmorrhages	Vary in size up to 1/- piece or several inches. Hæmorrhages present in fat and general musculature, and so numerous as to give the subcutaneous tissues a blotched appearance	Are general in distribution and vary from scattered petechiæ to large hæmatoma-like suffusions. They involve subcutaneous tissues, fat, and intramuscular connective tissues.
Heart	Hæmorrhages on serous surfaces in the myocardium and on all the valves	The hæmorrhages may be so numerous as to cover the entire serous surfaces.
Respiratory system	Hæmorrhages in nose and pharynx, rarely in larynx and trachea and not always in the lungs. Often many on the pleura and may be very large in the lungs	Common throughout the entire tract. Lungs may shew marked hypostasis.
Alimentary canal	Hæmorrhages in mouth, pharynx and gullet and a few in the first, second, and third stomachs. Numerous hæmorrhages in abdomen up to 1 cm. in diameter, not so numerous along the bowel but everywhere present. Blood-stained fluid in	Identical. In the abomasum very small ulcers were recorded. They were never numerous.

two per cent. solution of caustic soda, followed by liberal application of a lotion containing olive oil, methylated spirits, and sodium salicylate.

It was, however, at least three months before the hair grew again in profusion and the skin became clear of dandruff.

Summary.

1.—During the early months of 1929 a severe mortality occurred amongst imported pedigree bovines in the Nairobi Veterinary quarantine station.

2.—The disease was characterised by hæmorrhages into the skin, subcutaneous tissues, submucosæ, and one or more of the serous cavities.

3.—A noticeable symptom of the disease was the delayed clotting time of the blood. Owing to uncontrollable hæmorrhages into the various organs and tissues the animals rapidly died from loss of blood in untreated cases.

4.—The disease was noticed in animals which had been inoculated with the redwater-anaplasmosis vaccine. One case, however, was observed in an unvaccinated pregnant heifer, which contracted the disease a month after calving.

5.—The cause of the disease remains obscure. Bacterial, protozoan, and filter-passing viruses can be definitely excluded as the causal agent. The disease was not transmissible to other cattle or to horses, sheep, and rabbits.

6.—The mortality in untreated animals was 100 per cent.

7.—Adrenalin hydrochloride administered freely in large doses proved to be a specific.

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Appendix.

In this appendix the protocols dealing with transmission experiments are set out· n no instance were positive results obtained.

A.—Inoculation of Whole Blood from Sick Cases.

Date.	*Origin of Blood.*	*Animals Inoculated.*	*Amount of Blood Used.*
4/2/29	Heifer No. 27, obtained before death	Ox 3024	5 c.c. intravenously
4/2/29	Bull No. 20, obtained before death	Ox 2812	Ditto
7/2/29	Heifer No. 68, obtained before death	Ox 2777	10 c.c. intravenously
23/3/29	Bull No. 41, obtained before death	Ox 2493	70 c.c. intravenously
6/2/29	Heifer No. 68, obtained before death	Rabbit No. B	3 c.c. subcutaneously
6/2/29	Bull No. 37, obtained during life	Rabbit No. A	3 c.c. do.
7/2/29	Heifer No. 68, obtained during life	Sheep 3077 and 3042	3 c.c. subcutaneously into each animal
7/2/29	As above	Equine 1240	10 c.c. intravenously
12/2/29	Bull No. 42, bled during life	Oxen 2429 and 2432	25 c.c. intravenously in each case

B.—Inoculation of Laked Blood Passed per Berkefeld or Seitz Filter.

Date.	*Origin of Blood.*	*Animal Inoculated.*	*Amount of Blood Used.*
11/2/29	Bovine 66, bled during life	Ox 3274	10 c.c. intravenously
11/2/29	Bovine 68, bled during life	Ox 3238	ditto
11/2/29	Bovine 42, bled during life	Ox 3447	ditto
11/2/29	Pooled blood from bovines 42, 66, and 68, bled during life	Ox 3254	ditto

AN ATYPICAL FOWL-PLAGUE VIRUS FROM EGYPT.

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In 1929 Lagrange[1] reported a disease in fowls, due to a filter-passing virus, which caused heavy losses in Egypt. He considered it a new and, so far, unrecorded disease. Its differential diagnosis from the two other somewhat similar diseases of fowls caused by filter-passing viruses, Newcastle disease and fowl-plague, was based on the following findings :—

I. The Egyptian disease was said to differ from Newcastle disease, first described by Doyle[2] in 1927 and since then reported from the Dutch East Indies[3] and the Philippine Islands,[4] in the following points :—

(*a*) *Symptoms.*—There was rarely any marked respiratory trouble or the excessive salivation which is so characteristic of Newcastle disease.

(*b*) *Lesions.*—The sero-fibrinous exudate found in the sub-cutaneous tissues and around the heart of birds dead of the Egyptian disease was not present in Newcastle disease.

(*c*) *Infectivity of the Blood.*—The blood was never infective in less than 0·002 c.c.

(*d*) *Pathogenesis.*—The pigeon was not susceptible to this disease, whilst inoculation with Newcastle virus is almost invariably fatal.

II. The Egyptian Virus differed from that of fowl-plague, which incidentally is enzootic in Egypt, in the following points :—

(*a*) *Infectivity of the Blood.*—The infectivity of the blood in the Egyptian disease was low, whilst the virus content of the blood in fowl-plague is extraordinarily high.

(*b*) *Pathogenesis.*—The Egyptian disease attacked only fowls, and not pigeons and ducks.

A fowl infected with fowl-plague rarely takes more than 48 hours to die, whereas in the case of the Egyptian disease birds survived for four to eight days.

We take this opportunity of expressing our indebtedness to M. Lagrange, of the Conseil Sanitaire, Maritime et Quarantenaire d'Egypte, for supplying us with the Egyptian virus and enabling the following experiments to be carried out.

I. Period of Incubation.

Out of more than 100 fowls infected intramuscularly with the Egyptian virus, only three survived less than four days. This is in agreement with Lagrange's observation, whilst on one occasion an aged cock-bird died on the ninth day. These inoculations were carried out in series to the sixteenth passage without any appreciable change in the virulence of the virus. During the early passages it was considered possible that the long period of incubation might have been

due to a weakening of the virus through storage whilst in transit, or to the fact that the strain of fowls employed may have offered more resistance to the virus. The former idea was disproved, however, as the virus stabilised itself by killing fowls in an average period of four days when fresh material was used. Lagrange states that the period of incubation was stabilised through frequent passage at six days.

This observation would suggest that in this respect the Egyptian disease was similar to the Newcastle disease, which requires from four to fourteen days to kill a fowl by inoculation. In observations on a large number of fowls, inoculated with strains of fowl-plague from different sources, only in a few cases has it been found that disease required more than three days to kill a fowl, whilst usually death occurred within fifty hours. Seventeen out of twenty fowls, inoculated intramuscularly with a suspension of muscle from a fowl infected with fowl-plague (Palestine strain), died within forty-eight hours, and the average period of incubation in this experiment worked out at not more than 2·2 days.

From the results which follow, it will be noted that the combined periods of incubation of the Egyptian disease for twenty birds amounted to ninety days, and the average period was just over 4·5 days, that is, more than twice that of ordinary fowl-plague.

Passage and Incubation Period of the Egyptian Virus.

Passage.	*No. of Fowl.*	*Result.*	*Post-mortem.*	*Survived.*
1st	1122	Died	No Lesions	5 days
2nd	1058	,,	Great emaciation	4 ,,
3rd	1158	,,	Great emaciation	4 ,,
4th	1243	,,	Typical*	5 ,,
5th	R.I.R.C.	,,	Great emaciation	9 ,,
6th	1489	,,	Typical*	5½ ,,
7th	1505	,,	Typical*	4 ,,
8th	1498	,,	No P.M.	4 ,,
9th	1567	,,	Typical*	4 ,,
10th	1378	,,	No P.M.	3½ ,,
11th	1558	,,	Typical*	4 ,,
12th	1426	,,	No P.M.	4 ,,
13th	1771	,,	Typical*	3½ ,,
13th	1784	,,	Proventriculus only	5 ,,
14th	1682	,,	Typical*	5 ,,
14th	1684	,,	Typical*	5 ,,
14th	1661	,,	Proventriculus only	5 ,,
15th	299	,,	Typical*	4 ,,
16th	376	,,	Exudate around heart only	3 ,,

* Those fowls which on post-mortem revealed a reddish-purple discolouration of the skin, hæmorrhages of the proventriculus, petechiæ of any of the serous surfaces, and gelatinous exudate around the heart, were considered as having " typical " lesions of the Egyptian disease.

II. Symptoms.

Birds were usually found dead after four days without any symptoms having been manifested. The feathers were ruffled, and there

was a marked weakness of the legs in those fowls noted to be ailing. They sought a quiet dark corner to die, with the head away from the daylight. There was no appreciable discharge of exudate or escape of saliva from the nostrils or mouth in either naturally or artificially infected birds. In some cases diarrhœa was evident at or immediately before death.

These symptoms differ very definitely from those found in Newcastle disease. That characteristic dripping of stringy saliva from the beak was not present, neither could one hear the subdued croaking sounds made by a bird breathing through its open mouth while fighting for breath, as when dying from Newcastle disease. There were no nervous symptoms such as the motor paralysis found in a majority of birds suffering from Newcastle disease, and the fœtid diarrhœa noted in that disease was also absent.

In fowl-plague symptoms are rarely noted, owing to the very rapid course of the disease. In those showing symptoms a general malaise and inappetence is noted. The feathers may be ruffled, and the fowl dies with its head down, often resting it in a corner. Should a fowl be able to rest its head below the level of the rest of the body when dying œdema of the head rapidly supervenes. This has been noted in a number of cases, and may explain why Mohler,[5] Krohn,[6] Gerlach and Michalka,[7] Stubbs,[8] Beaudette,[9] and others, give œdema of the head as a lesion of fowl-plague, although Doyle[2] and other workers do not describe it.

III. Post-mortem Findings.

A comparative study of the post-mortem findings in fowls dead from the Egyptian disease and from fowl-plague has not revealed any noteworthy differences. In a few carcases no lesions could be found except a slightly purple discolouration of the skin, which is usual in cases of fowl-plague. The absence of lesions has also been noted occasionally in ordinary acute fowl-plague. A gelatinous œdema around the heart and in the subcutaneous tissues was often present. Petechial hæmorrhages on the mucous membrane of the proventriculus and on the serous surface of the sternum were almost invariably present. In some cases petechial hæmorrhages over the entire serous surfaces of the visceral organs have been observed.

The presence of slight exudate and saliva in the mouth and larynx was noted in this disease and also in fowl-plague, but could not in any way be compared with the large quantity often encountered in Newcastle disease. This slight exudate in the mouth of fowls dead of fowl-plague was first recorded by Leclainche[10] in 1904. It is, however, of no diagnostic value, as it is frequently found in fowls dead of other diseases.

Bacteriological examinations of the heart blood, brain, and spleen, performed immediately after the death of a number of fowls, were all negative. Cultures were made on agar and blood agar and in peptone broth, and incubated ærobically and anærobically at 37° C. for several days.

IV. Infectivity.

The blood was infective, and also the liver, spleen, muscle, brain, and spinal cord. The minimum infective dose of whole blood, taken on the second day of the disease, was 0·0025 c.c. On the fourth day the blood of the same fowl was infective in a quantity of 0·002 c.c., but not in smaller amounts. This bird died during the night of the ninth day after inoculation, and by the morning the blood was coagulated. Out of six fowls inoculated with dilutions made from some sero-sanguineous fluid obtained from the heart, only one died. This fowl had received 0·001 c.c., and it succumbed on the fourth day, revealing typical lesions of this disease. The remaining five fowls were proved to be susceptible to the Egyptian virus on subsequent testing.

In one case 0·25 c.c. blood failed to kill a fowl ; this fowl died after a second inoculation with virulent material given a week later. Lagrange[1] has recorded similar results, which he considers are due to " auto-sterilisation " as reported by Levaditi and his co-workers in reference to herpes, rabies, and the virus of encephalitis.

Our findings agree with those of Lagrange, but differ greatly from the usual figures given for fowl-plague. Todd[12] found the plasma virulent in doses of 0·0001 c.c., red cells in doses of 0·0001 c.c., and white cells in doses of 0·000001 c.c. given intramuscularly. Others[13] have been able to infect fowls with a dose of 0·000000001 c.c. of blood.

V. Pathogenicity.

Pigeons.—The pigeon is refractory to infection with fowl-plague. Freese[14] and Leclainche[10] failed to infect pigeons, although the last-named used very large doses of virus in his attempts. Maggiora and Valenti[15] succeeded only after starving a young pigeon, but failed with healthy young or adult pigeons. Kleine and Möller[16] succeeded in infecting only one nestling, although eight breeds of pigeons were used in their attempts. Nakamara and Kawamura[17] claim to have set up fatal fowl-plague in young pigeons by inoculation, or even by feeding and by cloacal contact with virulent material. Lagrange[1] points out that the virus he isolated did not infect pigeons, but does not mention the age of the pigeons used or the methods employed. Both of these are important points.

Experiments.

We have found that adult pigeons are insusceptible to the Egyptian virus either by intravenous or intramuscular inoculation.

Inoculation with Egyptian Virus.

No of Bird.	*Date* 1929.	*Material and Dose.*	*Method*	*Result.*	*Discharged* 1929.
1393 Adult pigeon	10/8	1 c.c. Fresh blood	I/V	Nil	19/8
1394 ,, ,,	,,	,, ,, ,,	I/M	Nil	19/8
1505 Adult fowl	,,	,, ,, ,,	I/M	Died 14/8	
1393 Adult pigeon	19/8	1 c.c. Liver suspension	I/V	Nil	17/9
1397 ,, ,,	,,	,, ,, ,,	I/M	Nil	17/9
1567 Adult fowl	,,	,, ,, ,,	I/M	Died 23/8	

In the next attempt, two young pigeons under ten weeks old were tested by the intravenous and intramuscular routes.

Inoculations with Egyptian Virus.

No of Bird.	*Date* 1929.	*Material and Dose.*	*Method.*	*Result.*	*Discharged* 1929.
1824 Young pigeon	23/8	0·5 c.c. Spleen suspension	I/V	Nil	17/9
1829 „ „	„	0·5 c.c. Spleen suspension	I/M	Nil	17/9
1379 Adult fowl	„	0·5 c.c. Spleen suspension	I/M	Died 26/8	25/9

Two pigeons which had received this virus were tested with Newcastle virus to ascertain if they had developed any degree of immunity against that disease.

Inoculation with Newcastle Virus.

No of Bird.	*Date* 1929.	*Material and Dose.*	*Method.*	*Result.*	*Date* 1929.
1393 Adult pigeon	17/9	1 c.c. Spleen suspension	I/V	Died	23/9
1824 Young pigeon	„	1 c.c. Spleen suspension	„	„	25/9
1640 Adult fowl	„	1 c.c. Spleen suspension	„	„	23/9

These experiments prove that pigeons inoculated with the Egyptian virus have had no protection conferred on them against Newcastle disease, and that the Newcastle and Egyptian viruses are immunologically distinct.

It is to be noted here that we have on a number of occasions failed to infect adult pigeons with massive doses of fowl-plague virus, using various methods of inoculation, but a few young pigeons have been fatally infected by massive intravenous doses. The virus from one of these pigeons was easily passaged by injecting its blood into a hen. On many other occasions it has been found impossible to infect even young pigeons.

On the other hand, inoculation with Newcastle virus has invariably killed young and adult pigeons in four to ten days. The results of Nakamura and Kawamura[17] are not in agreement with those of the majority of other workers on fowl-plague, and would suggest that they were working with a virus more akin to that of Newcastle disease. They failed to find constantly any appreciable virus in the blood ; the period of incubation and the symptoms they describe are also more suggestive of Newcastle disease.

Ducks.

Freese[14] remarks that although ducks are said to be susceptible to natural infection with fowl-plague he had failed to infect them, using varied methods, and he knew of no one who had succeeded. Since then Miessner and Berge[18] have worked with the virus of fowl-plague, and suggested that it should be called " Poultry-plague " (Geflügelpest), as it affects pigeons, turkeys, geese, ducks, etc. They failed, however, to infect ducks, but quote Baudet[19] as

their authority. From the literature on the disease we are left with grave doubt as to whether the duck is or is not susceptible to fowl-plague virus.

Doyle[2] records infecting fatally one out of two ducks by the intravenous inoculation of mouth exudate from a Newcastle infected bird.

Experiments.

Two young Indian Runner ducks were inoculated respectively intravenously and intramuscularly with spleen suspension of Newcastle virus. Both ducks remained healthy. Another duck from the same brood resisted an intravenous inoculation with the Egyptian virus. After a long period of rest, these three ducks were inoculated with the Palestine strain of fowl-plague virus and again resisted infection.

No. of Bird.	*Date* 1929.	*Material and Dose.*	*Method.*	*Results.*	*Discharged* 1929.
Inoculation with Newcastle Virus :					
Duck 1600	12/9	1 c.c. Spleen suspension	I/V	Nil	31/9
Duck 1601	,,	,, ,, ,,	I/M	,,	31/9
Fowl 1553	,,	,, ,, ,,	I/V	Died 16/9	
Inoculation with Egyptian Virus :					
Duck 1599	23/9	1 c.c. Spleen suspension	I/V	Nil	5/10
Fowl 1771	,,	,, ,, ,,	I/M	Died 26/9	
Inoculation with Palestine Virus (Fowl-Plague) :					
Duck 1599	27/11	2 c.c. Spleen suspension	I/M	Nil	16/12
Duck 1600	,,	,, ,, ,,	S/C	Nil	16/12
Duck 1601	,,	,, ,, ,,	I/V	Nil	16/12
Fowl 1831	,,	0·2 c.c. Spleen suspension	I/M	Died 29/11	

Pheasants.

Marcone[20] has described outbreaks of a virus disease in pheasants. He studied them and came to the conclusion that they were due to the virus of fowl-plague.

It has been possible to test this Egyptian virus on a young pheasant.

Experiment.

The pheasant was inoculated intravenously, and was visibly ill on the third day, eating only sparsely. On the fourth day it was humped up in a corner with its feathers ruffled. It died on the fifth day.

Post-mortem examination revealed petechial hæmorrhages of the proventriculus and sero-gelatinous fluid around the heart, such as is often encountered in fowls dead of this virus.

Inoculation with Egyptian Virus.

No. of Bird.	*Date* 1929.	*Material and Dose.*	*Method.*	*Results* 1929.
Pheasant 1759	23/9	0·5 c.c. Spleen emulsion	I/V	Died 28/9
Fowl 1771	23/9	1·0 c.c. Spleen emulsion	I/M	Died 26/9

VI. Contact Infection.

It is an interesting fact that although fowl-plague is such a deadly disease and may run a rapid course through a flock during natural outbreaks, particularly in warm countries, experimentally it is difficult to pass it on by contact. In a recent outbreak in this country recorded by Hole,[12] although the disease did not run a very rapid course the mortality was nearly 100 per cent. in the infected stock.

This difficulty with contact experiments has been encountered by nearly all those who have worked on this question. If a healthy fowl is placed in contact with one that has been experimentally inoculated infection results in only a small percentage of cases. This is surprising when one considers that the excreta of diseased fowls are infective, and that a drop of such material dropped on to the cloaca of a healthy fowl sets up infection although no mucous membrane be broken or injured. This has been shown to be the case by Todd.[12]

Some light has been thrown on this problem by Doyle,[2] who showed that if the *amount* of infection be raised the probability of infecting a healthy contact is also increased. Thus, if four healthy fowls were placed in contact with four that had been inoculated infection resulted in each case.

Todd[12] advanced the theory that if birds were of different sexes the possibility of passing on the disease might be increased. This, he considered, would be feasible, especially during the breeding season.

Lagrange[1] notes in connection with the Egyptian virus that " La contagion extrême par contact direct, est arrêtée par la moindre barrière." On the other hand, with Newcastle disease a contact bird rarely, if ever, escapes infection.

Experiments.

Twelve pairs of hens were placed in separate fowl-coops, and one of each pair was infected by intramuscular injection with fowl-plague. Four (33 per cent.) of the healthy contacts died of fowl-plague.

Similarly, a dozen pairs of fowls have been tested with the Egyptian virus. Four contact fowls died. Those that died respectively on the fifth, eighth and twelfth days after contact showed typical lesions of this disease. The fourth died on the fourteenth day, but no lesions of the disease were found on post-mortem examination. The lungs showed extensive pneumonia.

It would appear from these few experiments that the mortality with the Egyptian virus after such limited contact is 25 per cent. This is very similar to the results obtained with fowl-plague virus.

VII. Cross-Immunity.

Owing to the difficulty of immunising birds against fowl-plague and also against the Egyptian disease, it has been possible to carry out only limited cross-immunity experiments.

Four birds, one immune to Newcastle disease, one immune to fowl-plague, and two healthy controls, were inoculated with the

CONTACT EXPERIMENTS.

No. of Fowl.	*Date 1929.*	*Remarks.*	*Results.*	*Duration of Experiment.*	*Post-mortem Findings.*	*Passage.*
1498	14/8	I/M	Died	4 days	No P.M.	8th
1571		Contact	Nil	26 ,,	—	—
1567	19/8	I/M	Died	4 ,,	Typical	9th
1527		Contact	Died	8 ,,	Typical	10th
1379	23/8	I/M	Died	3½ ,,	No P.M.	10th
1333		Contact	Nil	25 ,,	—	—
1558	27/8	I/M	Died	4 ,,	Typical	11th
964		Contact	Died	7 ,,	Typical	12th
1796	12/11	I/M	Died	4 ,,	Typical	13th
26		Contact	Nil	21 ,,	—	—
1784	12/11	I/M	Died	5 ,,	Only proventriculus	13th
1694		Contact	Died	12 ,,	Only gizzard	14th
1682	16/11	I/M	Died	5 ,,	Typical	14th
1683		Contact	Nil	26 ,,	—	—
1684	16/11	I/M	Died	5 ,,	Typical	14th
1671		Contact	Nil	26 ,,	—	—
1661	18/11	I/M	Died	5 ,,	Only proventriculus	14th
1663		Contact	Nil	24 ,,	—	—
1687	18/11	I/M	Died	4 ,,	Typical	14th
1693		Contact	Died	14 ,,	Pneumonia	—
280	12/12	I/M	Died	3 ,,	Typical	15th
337		Contact	Nil	19 ,,	—	—
299	12/12	I/M	Died	4 ,,	Typical	15th
341		Contact	Nil	19 ,,	—	—

Egyptian virus. The two healthy control birds and the Newcastle-immune died within five days. The plague-immune bird showed no ill-effects.

This virus has also been tested against a Palestine strain of fowl-plague and the Bax strain recently isolated in the South of England by Hole[21] at this laboratory.

INOCULATIONS WITH EGYPTIAN VIRUS.

No. of Fowl.		*Material.*	*Method.*	*Results.*
510	Newcastle Immune	1·5 c.c. Original Lagrange material	I/V	Died 5 days
1124	Healthy control	,,	I/V	Died 4 days
1122	,,	,,	I/V	Died 5 days
825	Plague Immune (Rice)	,,	I/V	Survived
955	Plague Immune (Palestine)	1 c.c. Spleen suspension	I/M	Survived
1158	Healthy Control	,,	I/M	Died 4 days
1178	Plague Immune (Bax)	,,	I/M	Survived
1239	Healthy control	,,	I/M	Died 5 days

Experiments.

After many failures, two hens were successfully immunised against the Egyptian virus by employing a bromoform virus vaccine. These hens were then inoculated with two strains of typical virulent fowl-plague, and they both resisted infection.

Inoculation with Viruses of Fowl-Plague from Different Sources.

No. of Fowl.		*Name of Virus.*	*Material.*	*Method.*	*Result.*
224	Egypt. virus immune	Rice	0·5 c.c. Spleen suspension	I/M	Survived
337	Healthy control	Rice	,,	I/M	Died 48 hrs.
225	Egypt. virus immune	Palestine	0·5 c.c. Liver suspension	I/M	Survived
341	Healthy control	Palestine	,,	I/M	Died 48 hrs.

After an interval of ten days Fowl 224, immune to the Egyptian disease, was inoculated with the Newcastle virus. It died of the typical disease in seven days, and thus showed that it had no protection against the latter disease.

Inoculation with Newcastle Virus.

No. of Fowl.		*Virus.*	*Material.*	*Method.*	*Result.*
224	Egypt. Immune	Newcastle	1·0 c.c. Liver suspension	I/V	Died 7 days
496	Healthy control	Newcastle	1·0 c.c. Liver suspension	I/V	Died 5 days

VIII. Filtration.

It is well known that the filterability of viruses varies considerably. It was with the object of finding out whether the Egyptian virus differs in this respect from that of ordinary fowl-plague that these experiments were undertaken.

Technique.

A fowl recently dead of Egyptian virus was opened, and the various tissues to be used were removed. Three to five grammes of the required tissue were then suspended in 20 c.c. of peptone broth. This suspension was first passed through glass wool, and then through a Chamberland L2. candle under a negative pressure of 760 mm. Hg. at room temperature. A 24 hours old culture of *B. avisepticus* in Martin's broth was used as a control to the filtration. A few loop-fulls of the filtrate were inoculated into a couple of tubes of Martin's broth, and inoculated ærobically for two days. A bacteriological examination of the blood also checked the filtration, and the susceptible hens did not die of fowl cholera.

Filtration Experiment with Egyptian Virus.

Carcase 1796.—Typical lesions and petechiæ on serous surface of sternum, and on the mucosa of the proventriculus. Died on the fourth day of the disease.

Carcase 1784.—This bird died on the fifth day of the disease. Proventricular petechiæ were the only lesions present.

The results of inoculating brain and spleen material from Fowls Nos. 1796 and 1784 are shown in Table I.

TABLE I.

No. of Fowl.	*Material Inoculated.*	*Dose.*	*Route.*	*Result.*	*Post-mortem Lesions.*
1685	Brain filtrate of 1796	4 c.c.	I/M	Died 7 days	Typical
1686	,, ,, ,,	4 c.c.	I/V	Died 7 days	Typical
1682	Unfiltered brain suspension	1 c.c.	I/M	Died 5 days	Typical
1659	Spleen and liver filtrate	3 c.c.	I/M	Died 4 days	Typical
1667	,, ,, ,,	2 c.c.	I/V	Died 4 days	Typical
1684	Unfiltered spleen and liver suspension	1 c.c.	I/M	Died 4 days	Typical
1678	Brain filtrate of 1784	2 c.c.	I/V	Died 4 days	Typical
1695	do.	4 c.c.	I/M	Died 5 days	Typical
1661	Brain unfiltered	0·5 c.c.	I/M	Died 5 days	No lesions
1657	Spleen and liver filtrate	2 c.c.	I/V	Survived	
1690	do.	4 c.c.	I/M	Survived	
1687	Spleen and liver unfiltered	1 c.c.	I/M	Died 4 days	Typical

Filtration Experiments with Fowl-Plague (Palestine Strain).

Similarly, filtration experiments which were carried out on two birds dead of typical virulent fowl-plague are embodied in Table II.

Carcase 325.—Died within 48 hours. Intense and numerous petechiæ present on all serous surfaces.

Carcase 334.—Died within 48 hours. There was considerable gelatinous œdema of the subcutaneous tissues and around the heart, but few petechiæ were present.

TABLE II.

No. of Fowl.	*Material Inoculated.*	*Dose.*	*Route.*	*Results.*	*Post-mortem.*
1688	Brain unfiltered of 325	0·5 c.c.	I/M	Died 2 days	Fowl plague
406	Brain filtrate	2 c.c.	I/M	Survived	
316	Brain filtrate	2 c.c	I/V	Survived	
326	Liver filtrate	3 c.c.	I/M	Died 2 days	Fowl plague
310	Liver filtrate	2 c.c.	I/V	Died 2 days	,,
323	Liver unfiltered	0·5 c.c.	I/M	Died 2 days	,,
371	Brain filtrate of 334	3 c.c.	I/M	Died 2 days	,,
1668	Brain filtrate	2 c.c.	I/V	Died 2 days	,,
396	Brain unfiltered	0·5 c.c.	I/M	Died 2 days	,,
395	Liver and spleen filtrate	2 c.c.	I/M	Died 2 days	,,
404	Liver and spleen filtrate	1 c.c.	I/V	Died 2 days	,,
400	Liver and spleen unfiltered	0·5 c.c.	I/M	Died 2 days	,,

All broth controls to these experiments showed no growth on incubating ærobically for two days at 37° C.

IX. Conclusions.

The following conclusions have been drawn from the experimental work reported in this paper :—

I. The Egyptian virus is distinct from that causing Newcastle disease in England, pseudo-fowlpest in the Dutch East Indies[3], and a similar disease in the Philippine Islands.[4] The results of work carried out in this laboratory and in the Philippine Islands have shown that these are one and the same virus.

SUMMARY OF RESULTS OBTAINED.

Virus.	*Period of Incubation.*	*Symptoms.*	*Lesions.*	*Pathogenesis.*				*M.L.D. of blood.*	*Contact Experiments*	*Cross-Immunity.*	*Recovery from artificial infection.*
				Fowls.	*Ducks.*	*Pigeons.*	*Pheasant.*				
Egyptain virus	Min. 3 days Ave. 4-5 days Max. 9 days	Often none observed Malaise Weakness of legs Diarrhœa in some cases just before death	May be none present Petechiæ on all serous surfaces Gelatinous exudate Small discrete petechiæ on proventricular mucosa Enteritis occasionally present	+	—	Young —. Old —.	+	0·001 c.c.	25%	With Fowl-plague present, with Newcastle none	None
Fowl-Plague Virus	Min. 1 day Ave. 1½-2½ days Max. 4 days	Often none observed Malaise Diarrhœa in some of the less acute cases	May be none present Petechiæ on all serous surfaces and in proventriculus Enteritis in a few cases. Sero-gelatinous exudate	+	—	Young —, + Old —.	+ (Marcone)	0·000001 c.c. and on several occasions less	35%	With Egyptian virus present, with Newcastle none	None
Newcastle Virus	Min. 3 days Ave. 5 days Max. 17 days (Doyle)	Excessive salivation Respiratory symptoms Fœtid diarrhœa in most cases Paralysis in many cases	May be none present Rarely petechiæ on sternum Petechiæ of serous surfaces when present more discrete and fewer Larger petechiæ on proventriculus Excessive mucus in mouth	+	— (Picard 2+ 3 —)	Young + Old +.	Not tested.	0·00025 c.c.	100%	With Fowl-plague none with Egyptian virus none	1% or less

(*a*) *Symptoms.*—Fowls affected with the Egyptian disease never present any excessive salivation or the subdued croaking often encountered in Newcastle disease.

(*b*) *Pathogenicity.*—The Egyptian virus is not pathogenic for pigeons.

(*c*) *Immunologically* the Egyptian virus is distinct from Newcastle virus.

(*d*) *Contact experiments* were successful in only 25 per cent. of the twelve pairs tested. From personal observations made on a number of occasions, when using the Newcastle virus a contact has never been known to escape infection.

II. The Egyptian virus may be said to differ from ordinary fowl-plague in the following two ways :—

(*a*) *The period of incubation*, which in the Egyptian disease is over four days, is more than twice that of ordinary fowl-plague.

(*b*) *The infectivity of the blood* is low, the highest M.L.D. being 0·001 c.c., whilst in fowl-plague a M.L.D. of 0·00001 c.c. is common, and in some cases a millionth of c.c. or less is infective.

III. That this disease is in reality a form of fowl-plague, although running a slower course and showing the virus in less concentration in the blood, is the obvious conclusion after noting the following important points of similarity :—

(*a*) *Immunologically* it has been tested against a number of fowl-plague viruses, and complete protection has always been obtained in the cross-immunity tests.

(*b*) *Symptoms*, when present, differed from those of fowl-plague only in being more prolonged.

(*c*) *The lesions* noted in the Egyptian disease were similar to those found in fowl-plague.

(*d*) The results of *filtration experiments* with the Egyptian virus were very similar to those obtained with fowl-plague virus.

(*e*) *Contact experiments* with the Egyptian virus revealed that only 25 per cent. of the pairs showed transmission, a result which is very similar to that obtained with the fowl-plague virus.

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